

FIG.1A

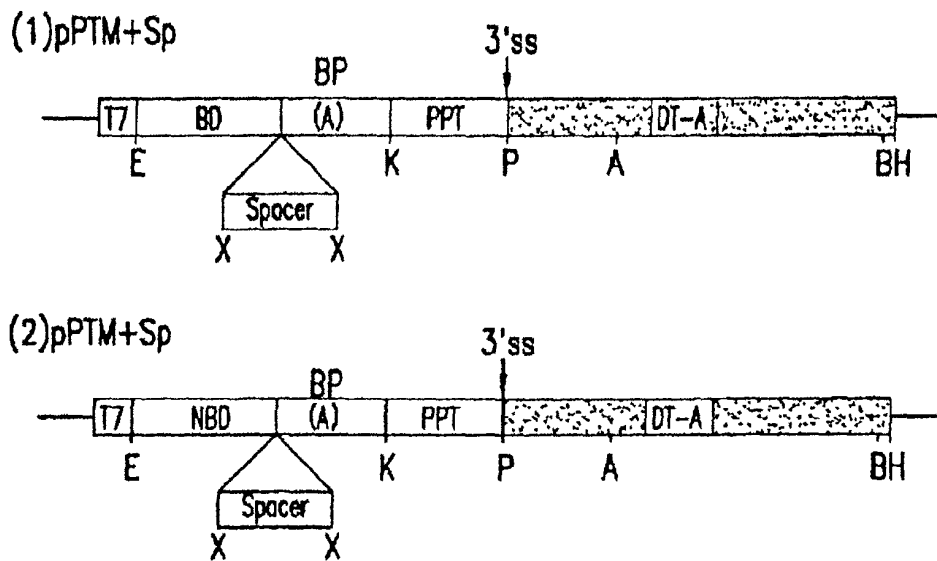


FIG.1B

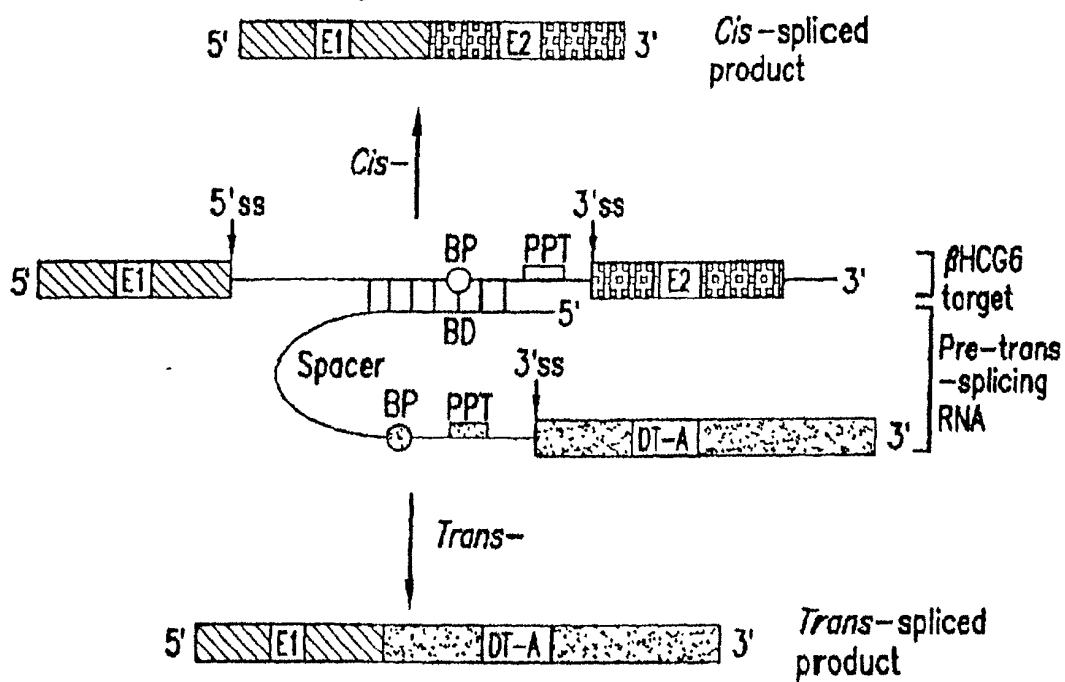


FIG.1C

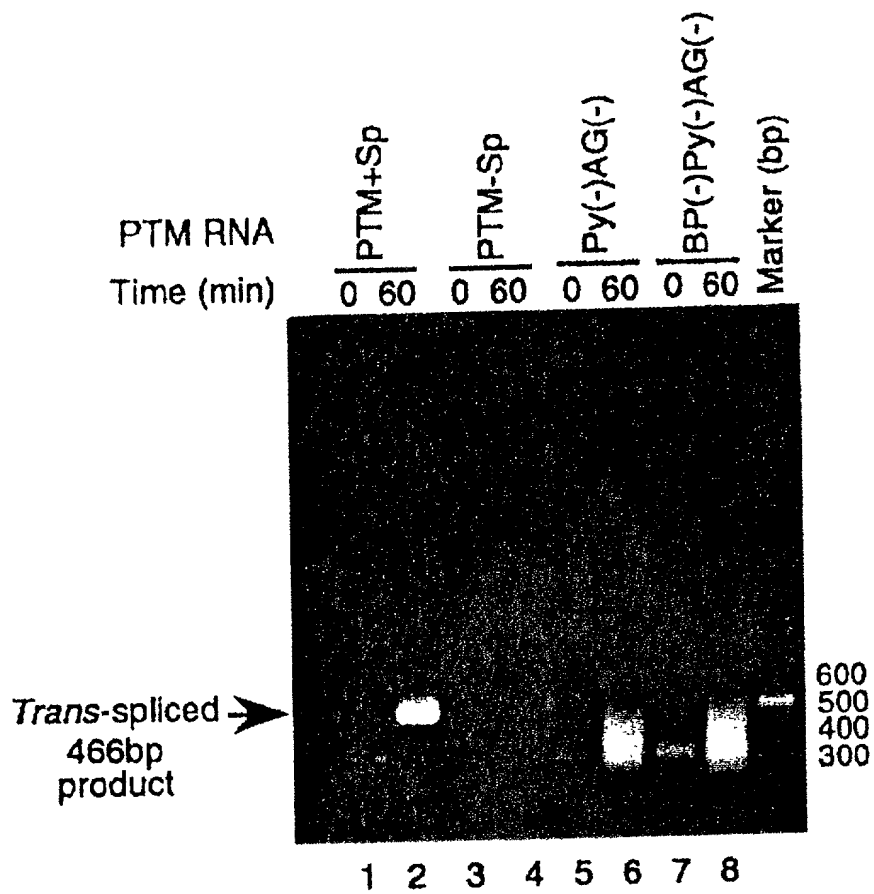


FIG.2A

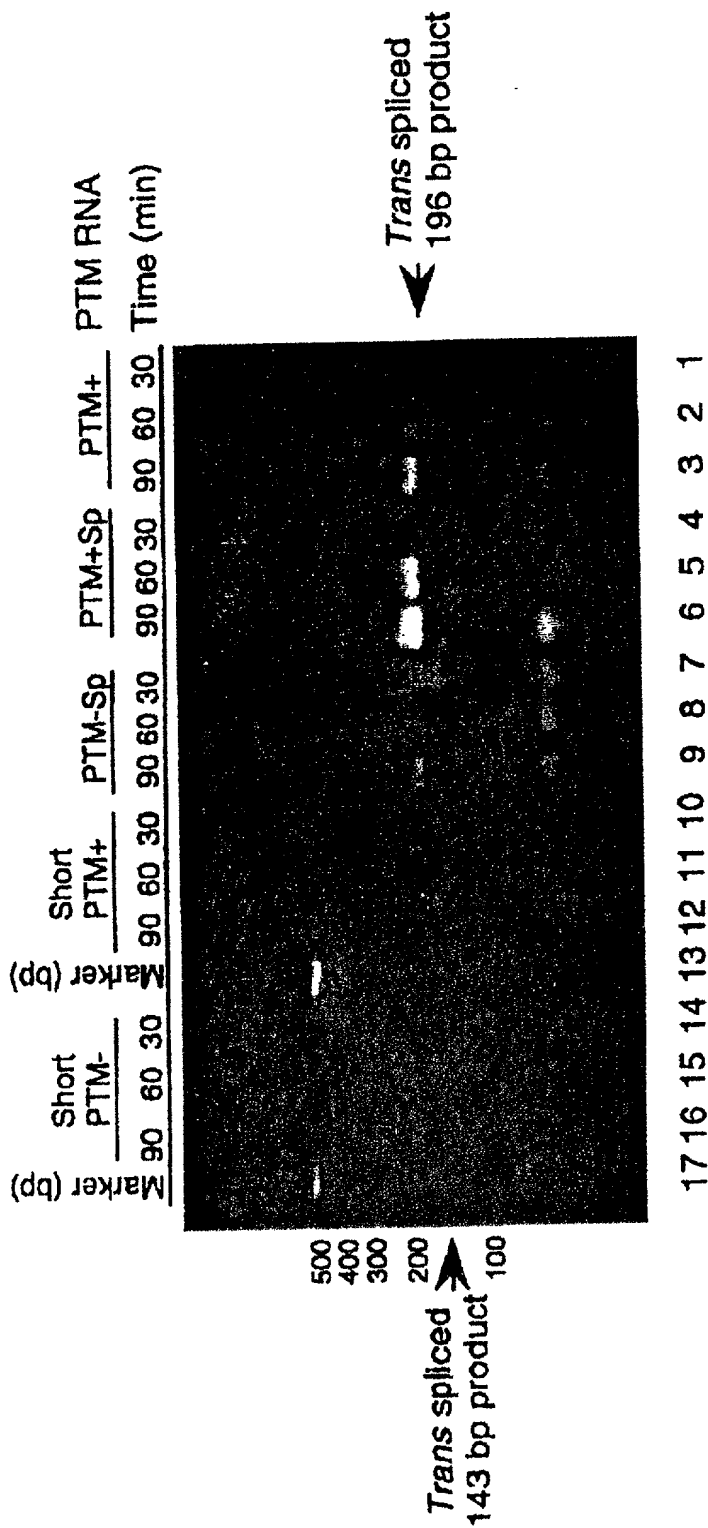


FIG.2B

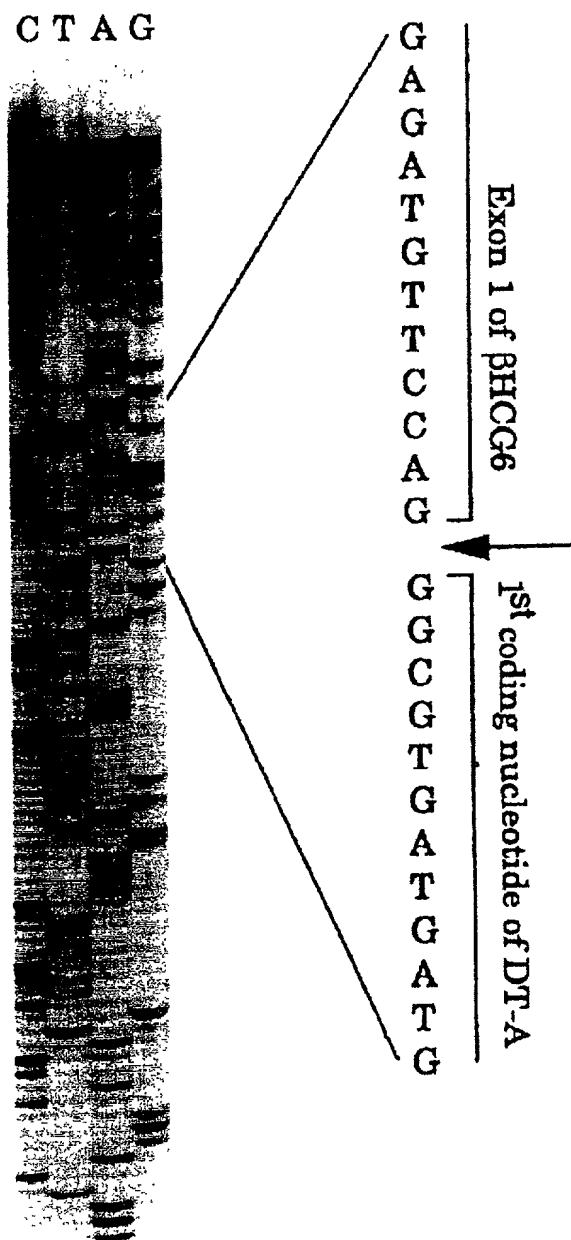
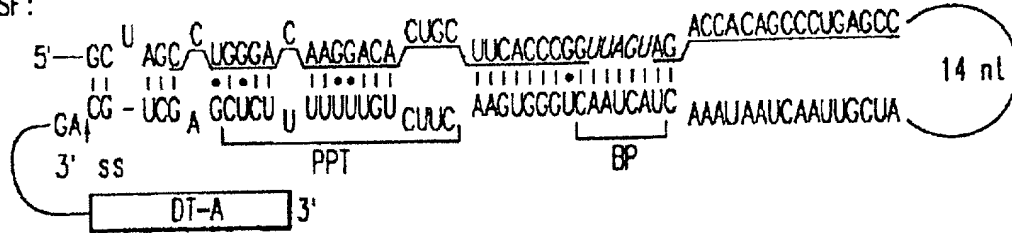


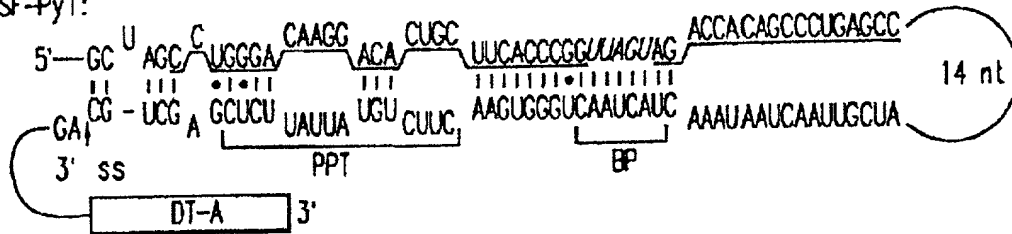
FIG.3

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1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

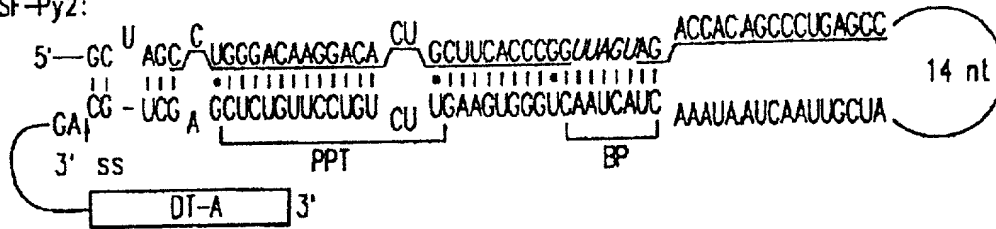


FIG.4A

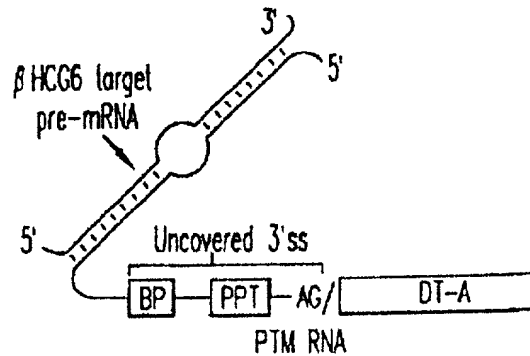


FIG.4B

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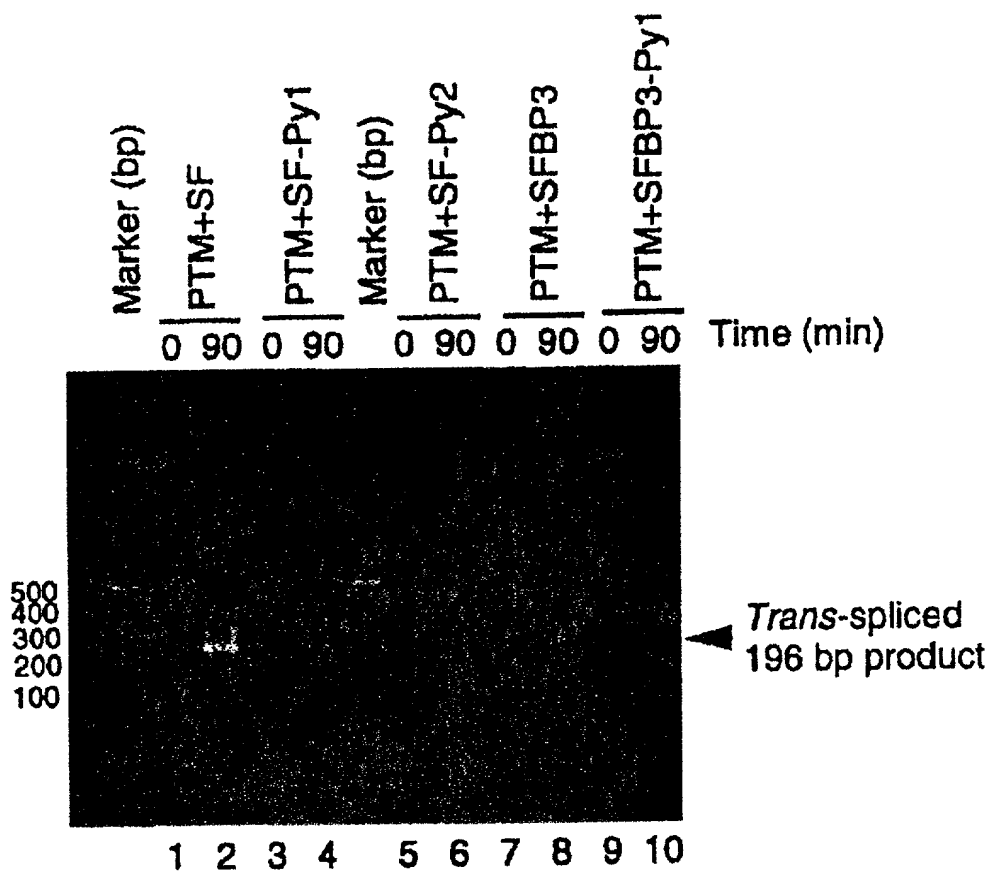


FIG.4C

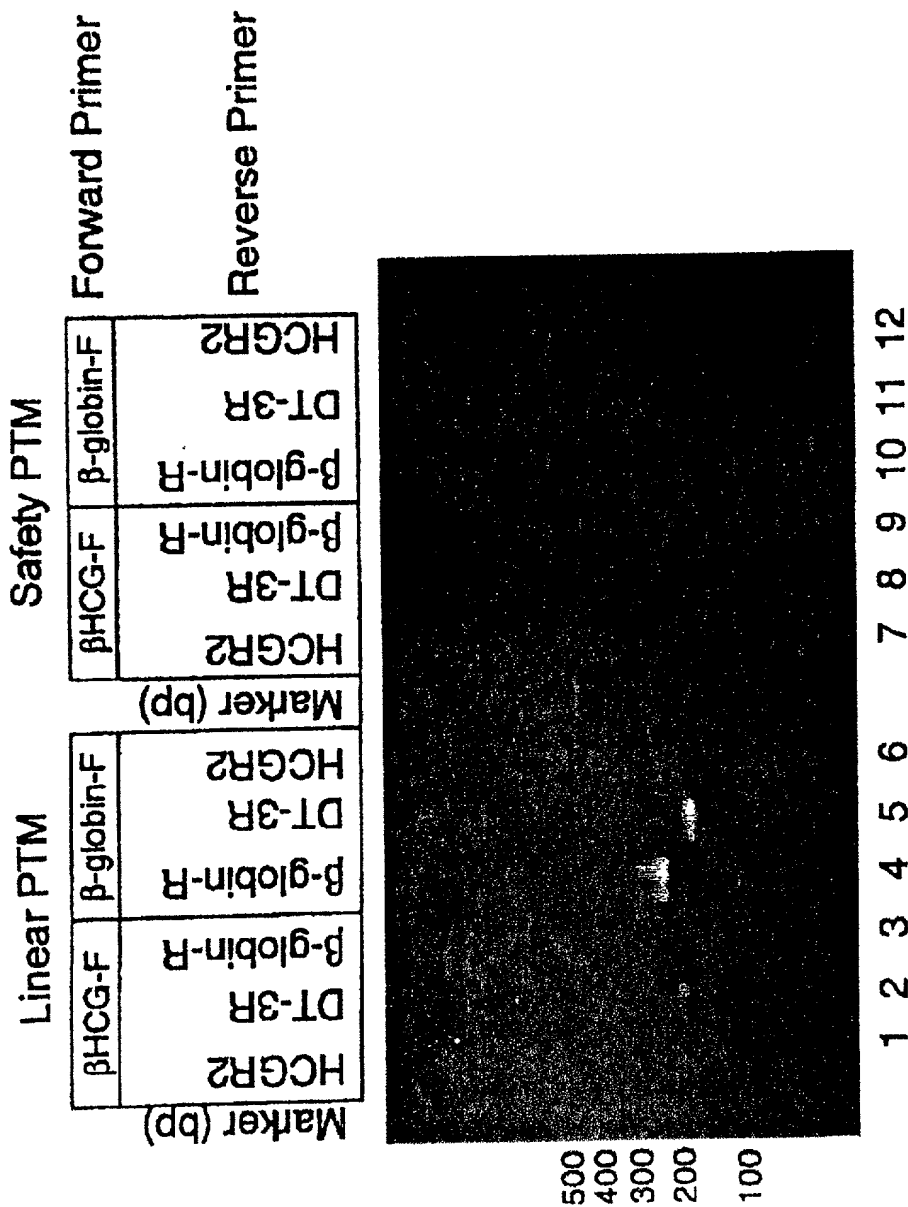


FIG.5



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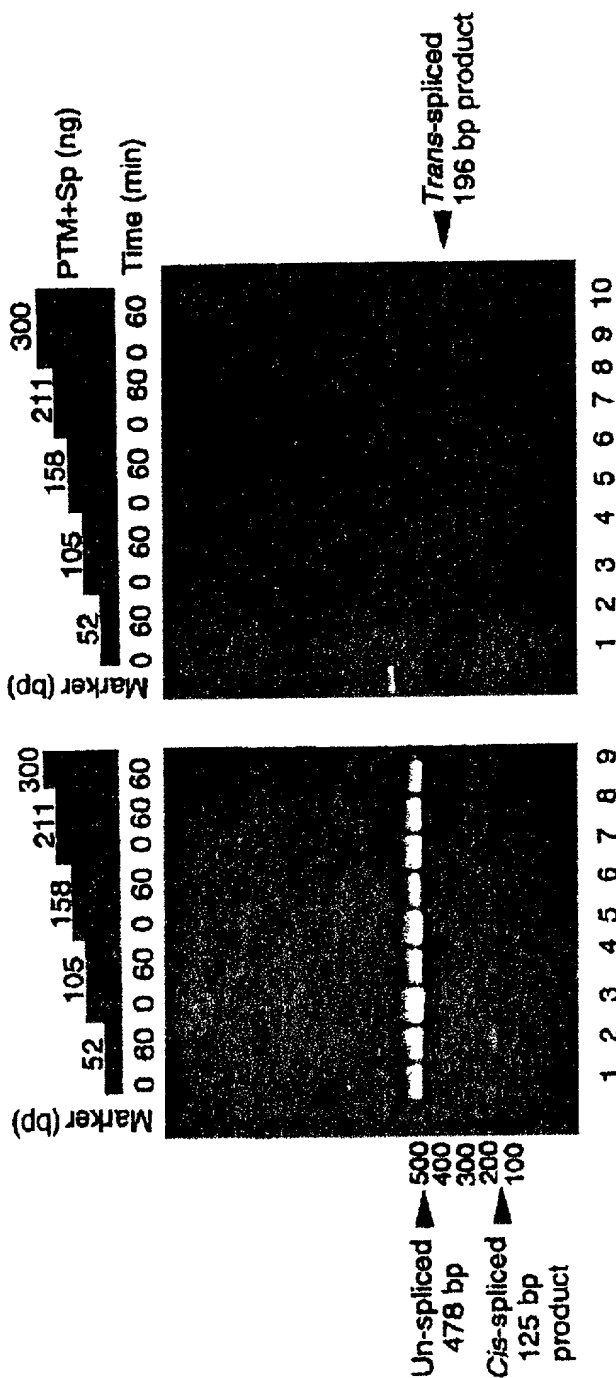


FIG. 6B

FIG. 6A

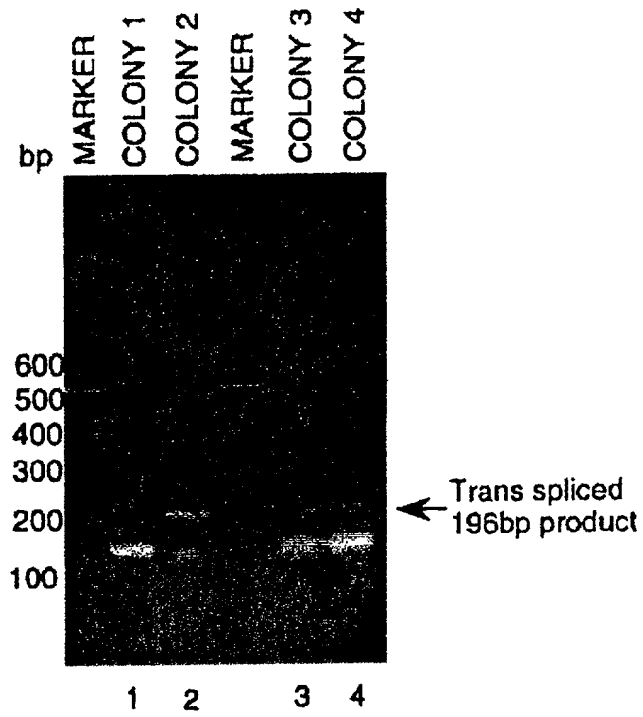


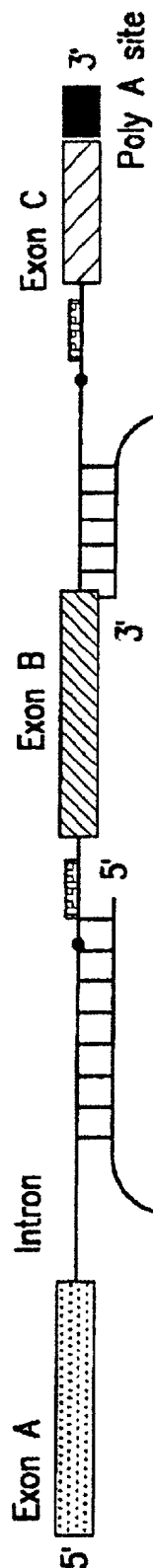
FIG.7A

EXON 1 OF  $\beta$ HCG6 ↓  
 5'-CAGGGACGCACCAAGGATGGAGATGTTCCAG-GGGCTGATGATGTTGTT  
 ↓ 1ST CODING NUCLEOTIDE OF DT-A  
 GATTCCTTAAATCTTTTGATGGAAACTTTCTTCGTACCAACGGGACTA  
 AACCTGGTTATGTAGATTCCATTCAAAA-3'

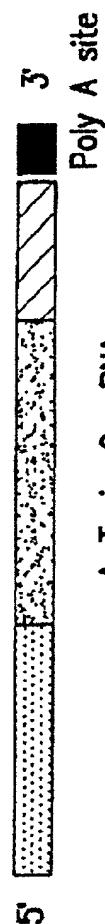
FIG.7B



*Cis*-splicing



*Trans*-splicing

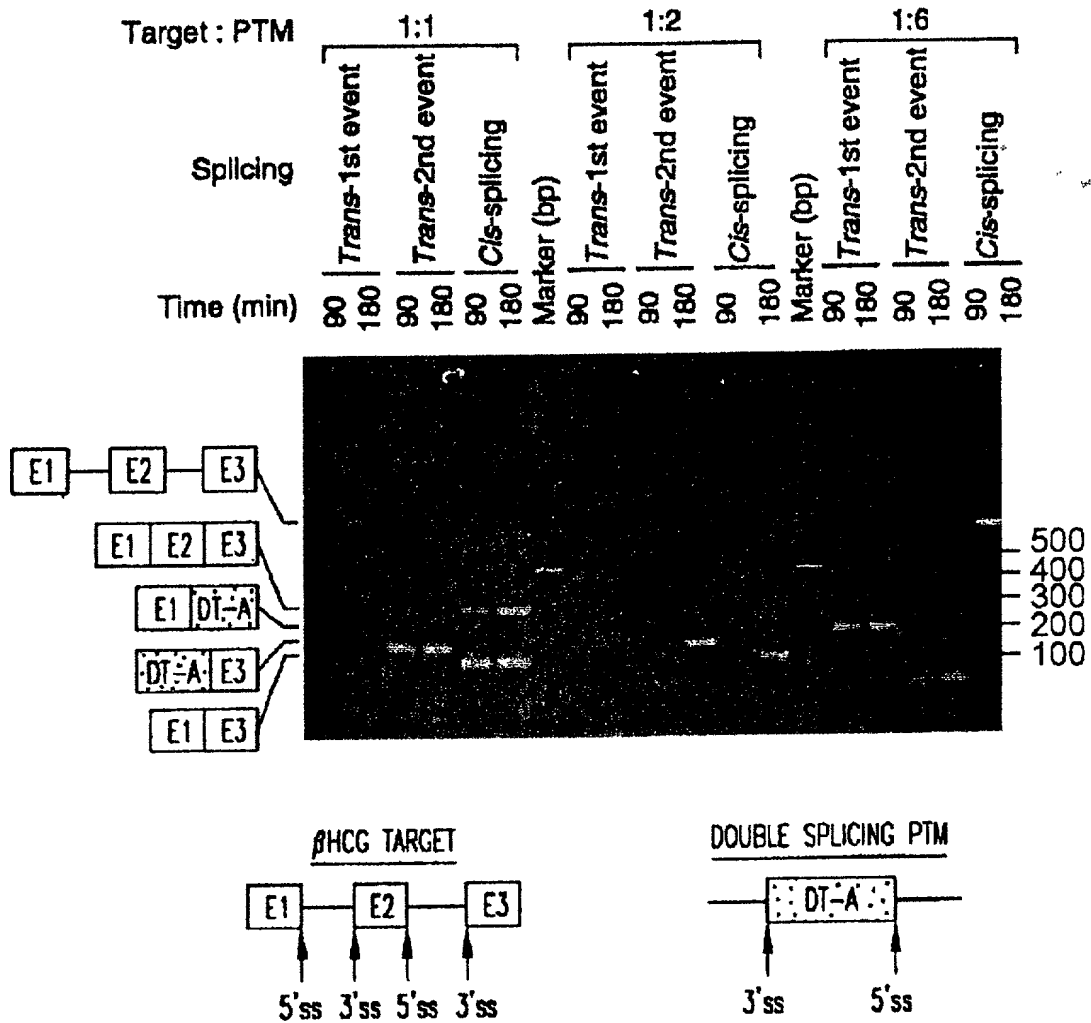


A-Toxin-C mRNA

FIG.8A

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Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

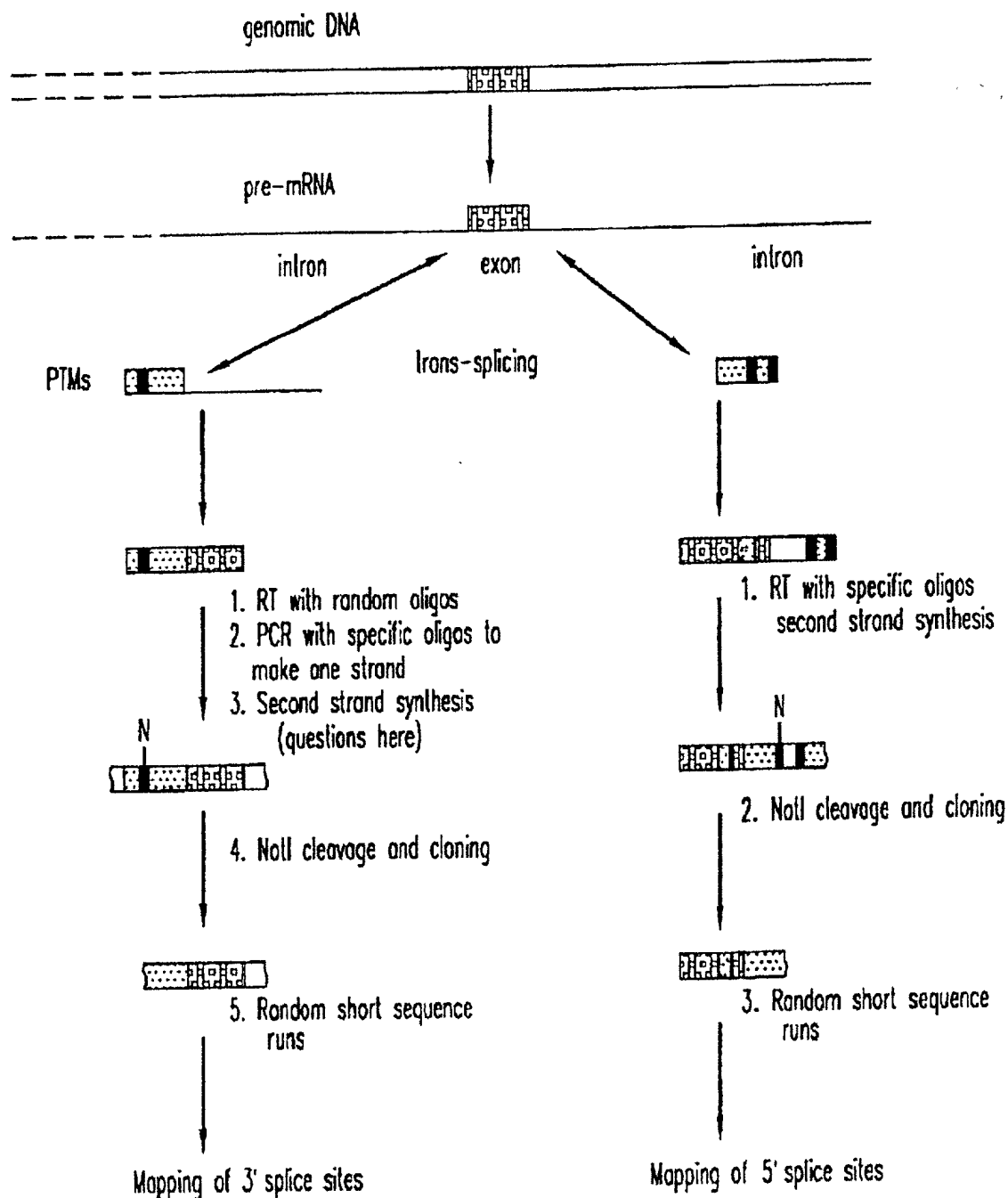


FIG.9



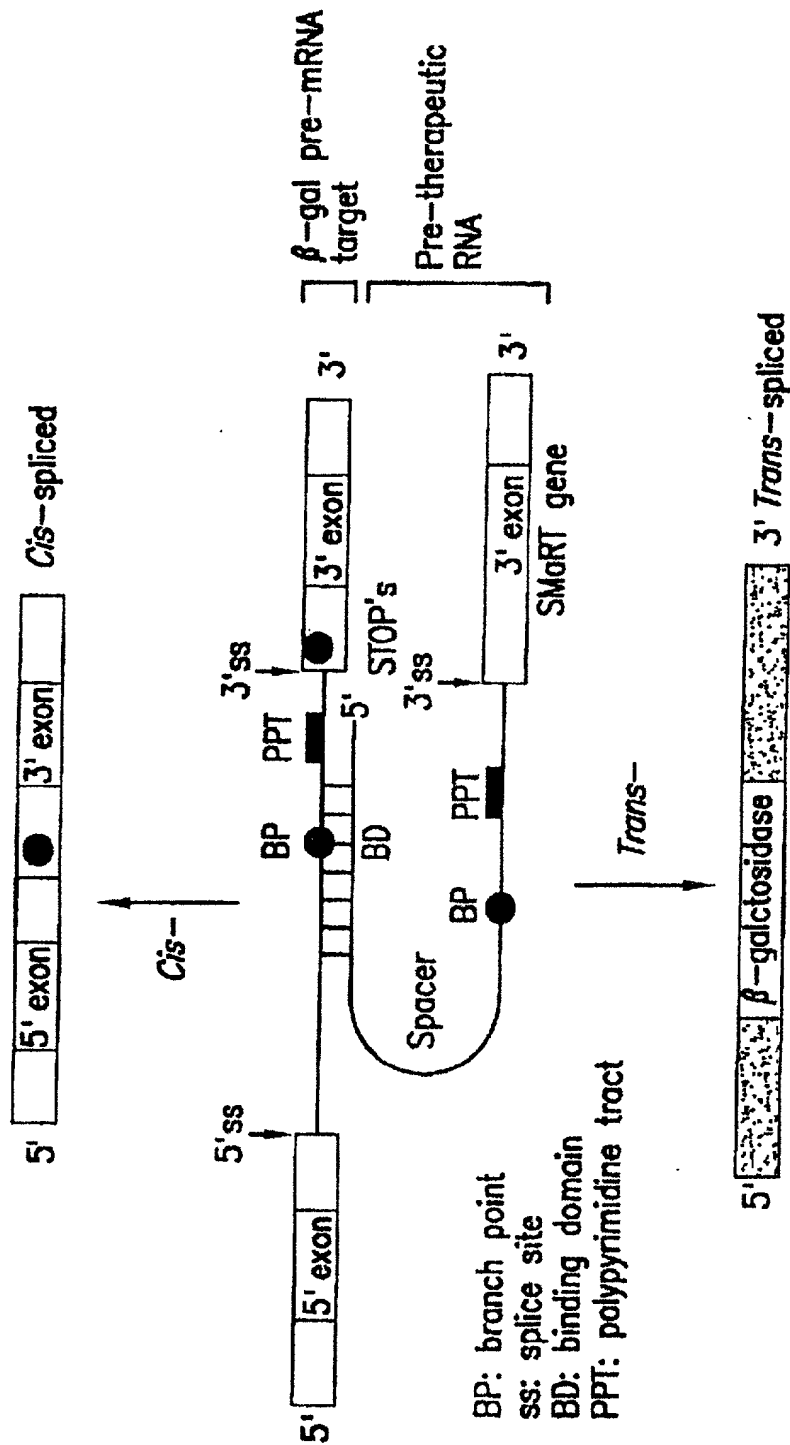


FIG.10B

16 8 91



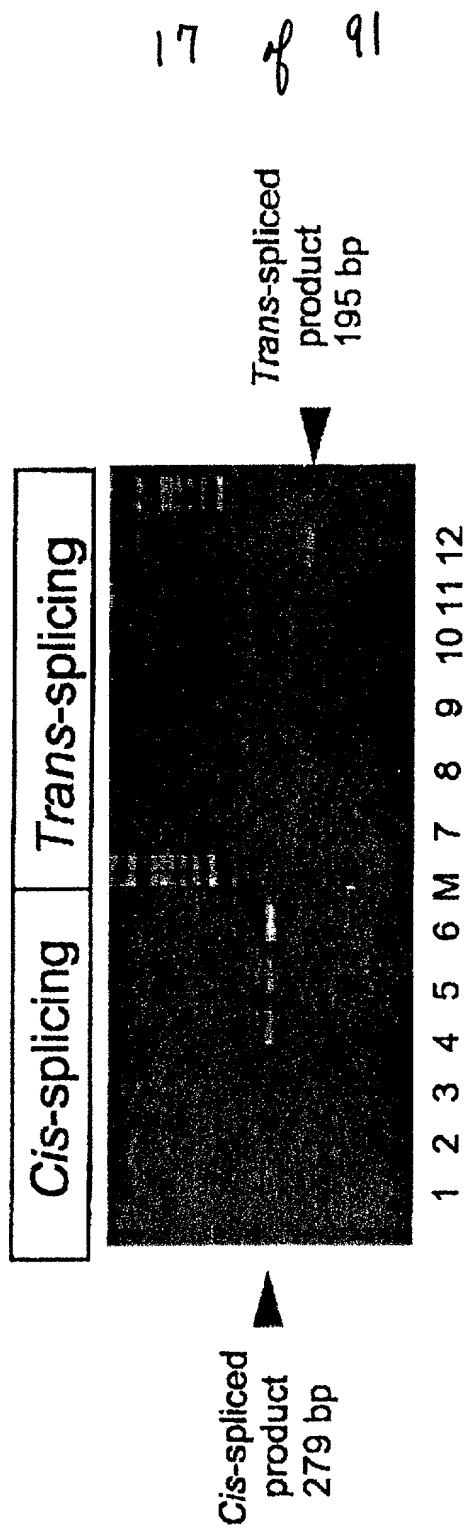


FIG.11A

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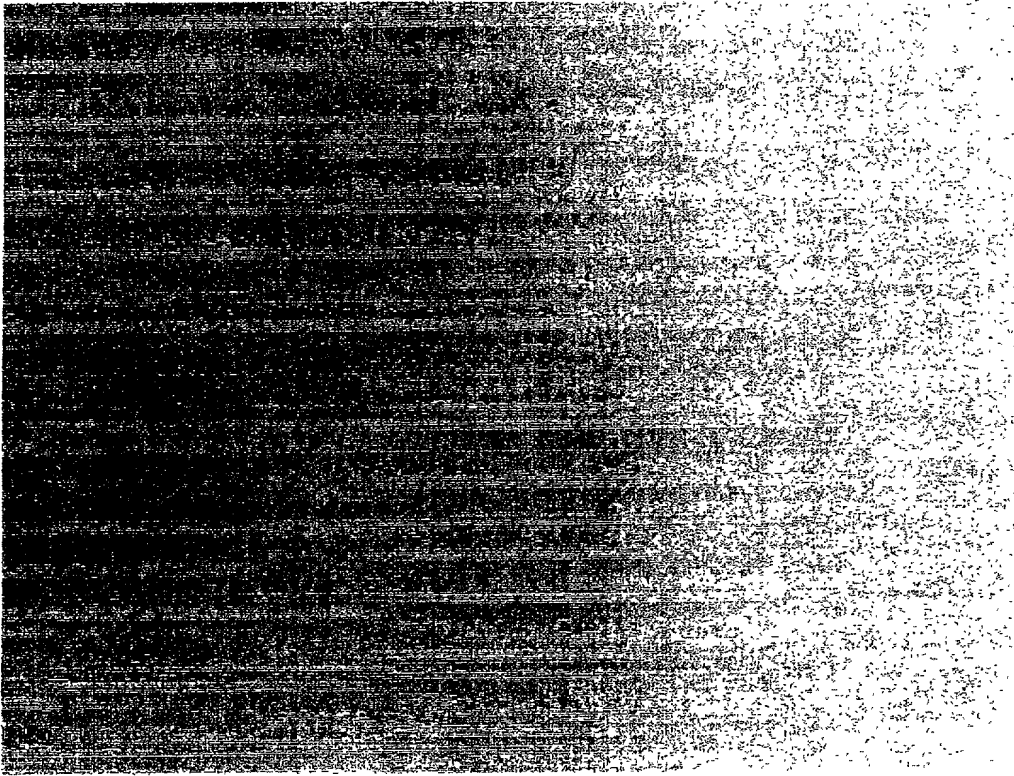


FIG.11B

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09041492 040402

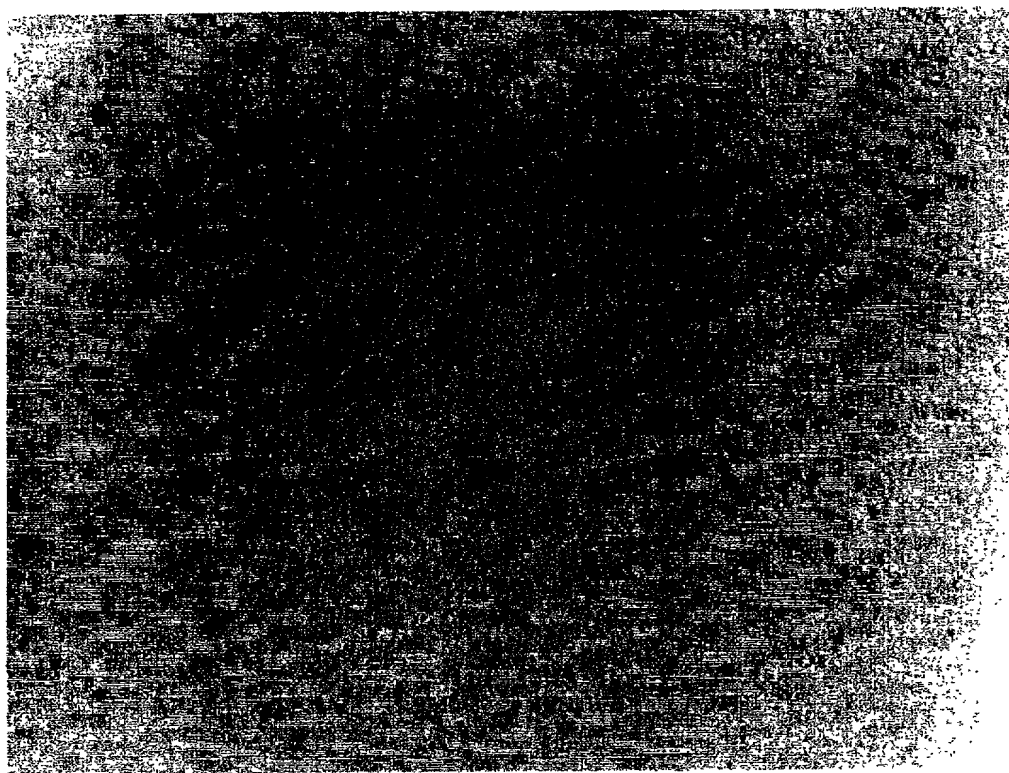


FIG.11C

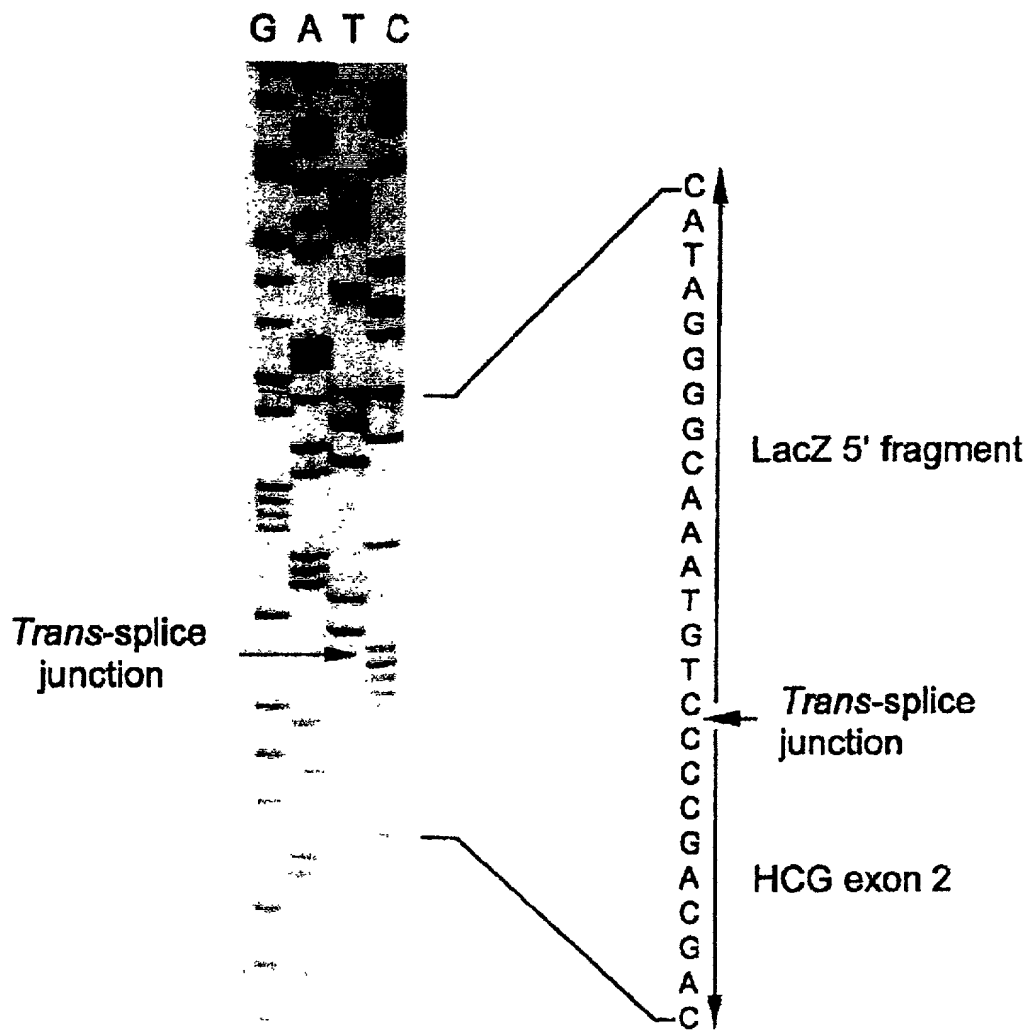


FIG.12A

### 1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

# Biolac-TR1

GGCTTTGGCTACCTGGAGAGACGGCCCGCTGATCCTTTGGCAATACGCCCAACGGCATGGGTAAACAGTCTTG

## Splice junction

GGGGTTTCGGCTAAATACTGGCAGCGTTTCGTCAGTATCCCGGTTTACAG/GGGCGCTTCGTCATAATG

GGACTCGGTGGATCAGTCGCTGATTAAATATGATGAAACGGCAACCGTGGTCGGCTTACGGCGGTGATTT

**Lac-TR2**

TGGCGATACGCCGAACGATCGCCAGTTCCTGATGAACGGTCTGGTCTTTGGCGACGGACGGCGCATCCAG  
LAC-INZ

## 2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

Biolac-TR1

GGCTTTTCGCTACCTGGAGAGACGGGCCCGCTGATCCTTTGGCAATACGCCACGCCGATGGGTACACAGCTCTGG

## Splice junction

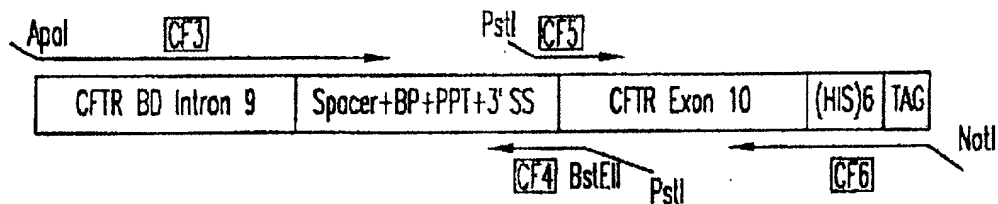
CGGTTTCGGCTAAATACITCGCAGCGTTTCGTCAGTATCCCGTTTACAG/CGGCTGCTGCTGTTGCTGCTGCT

## HCCR2

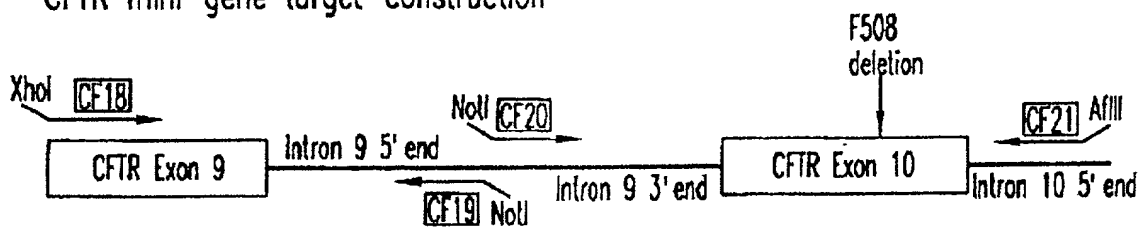
GAGCATGGCGCGGACATGGGCATCCAGGAGCCACTTCGGCCACGGTGGCG

**FIG. 12B**

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

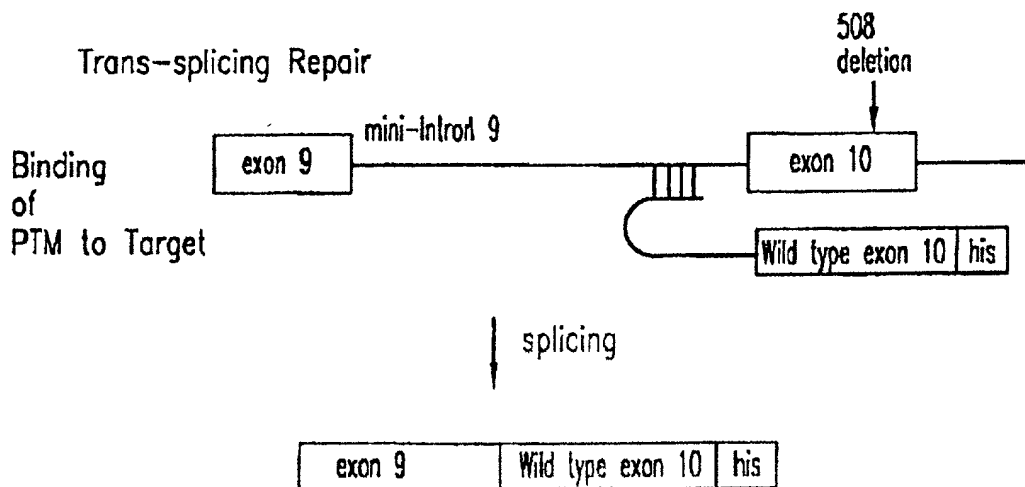


FIG.13

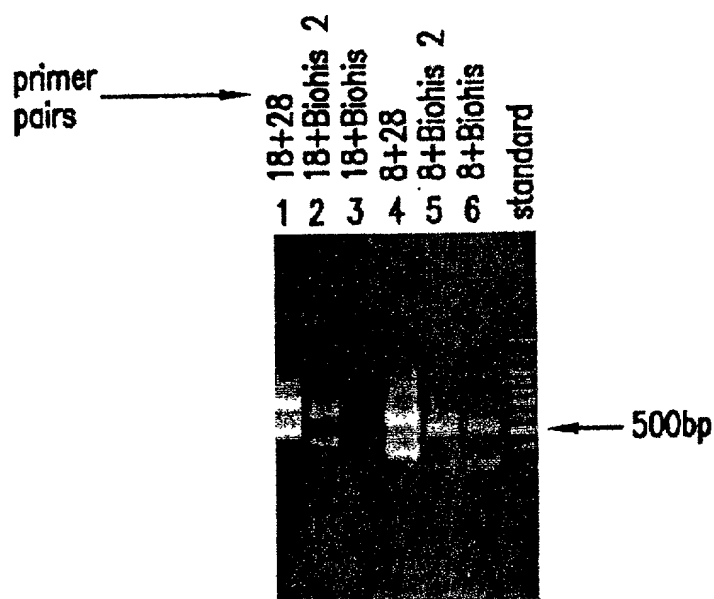


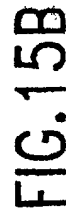
FIG.14

### Positions of Restriction Endonucleases sites (unique sites underlined)

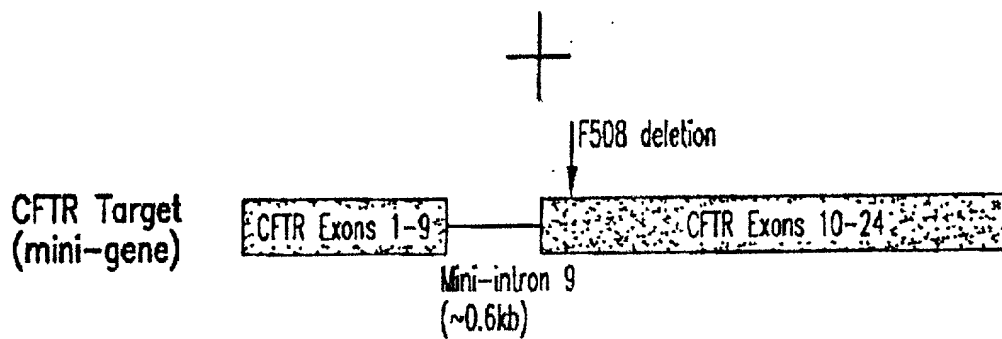
[illegible]

**FIG. 15A**





PTM CFTR BD Intron 9 Spacer+BP+PPT+3' SS CFTR exons 10-24 (His) 6 TAG



Cotransfect PTM and target molecules in HEK 293 cells and detect repaired CFTR mRNA by RT-PCR.

Repaired CFTR mRNA

CFTR Exons 1-9 Exons 10-24 CFTR (His) 6 TAG

FIG.16

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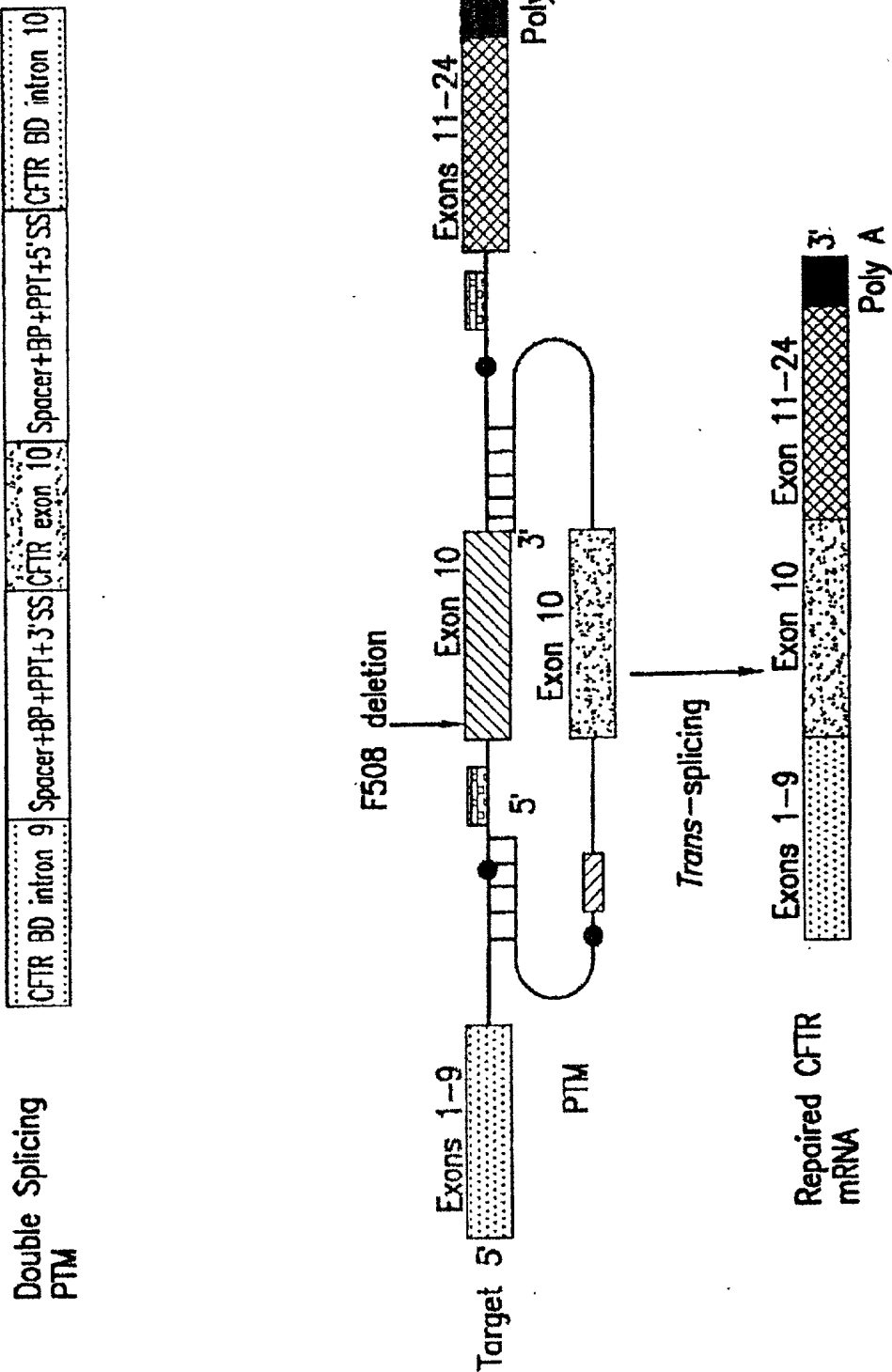
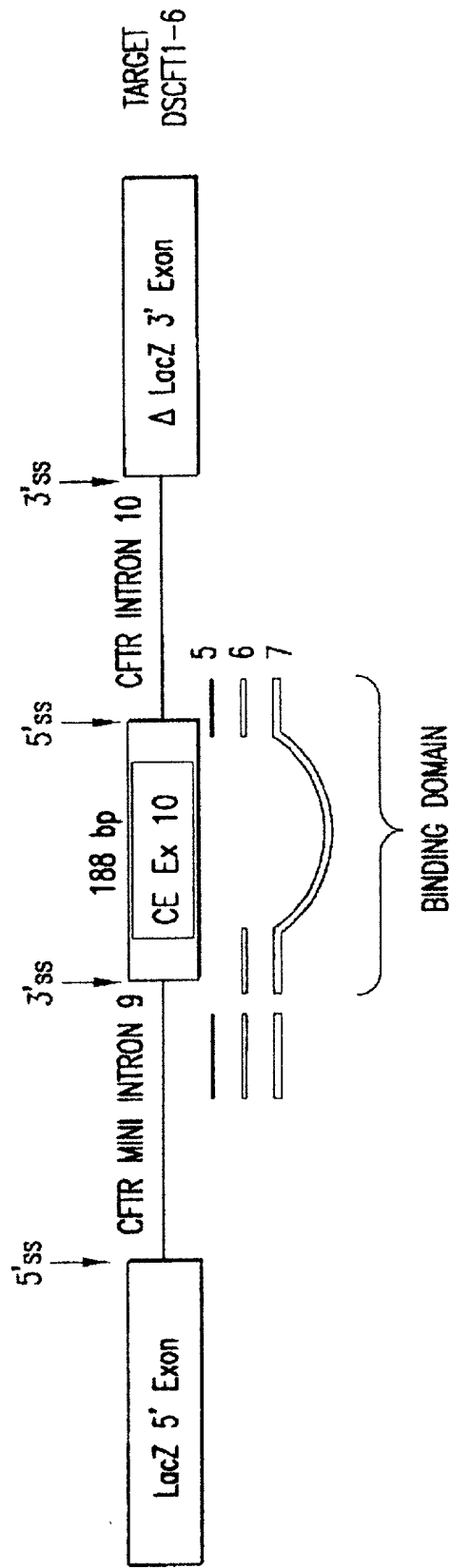


FIG.17

DOUBLE TRANS-SPLICING SPECIFIC TARGET



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FIG.18

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# DOUBLE TRANS-SPLICING PTMS

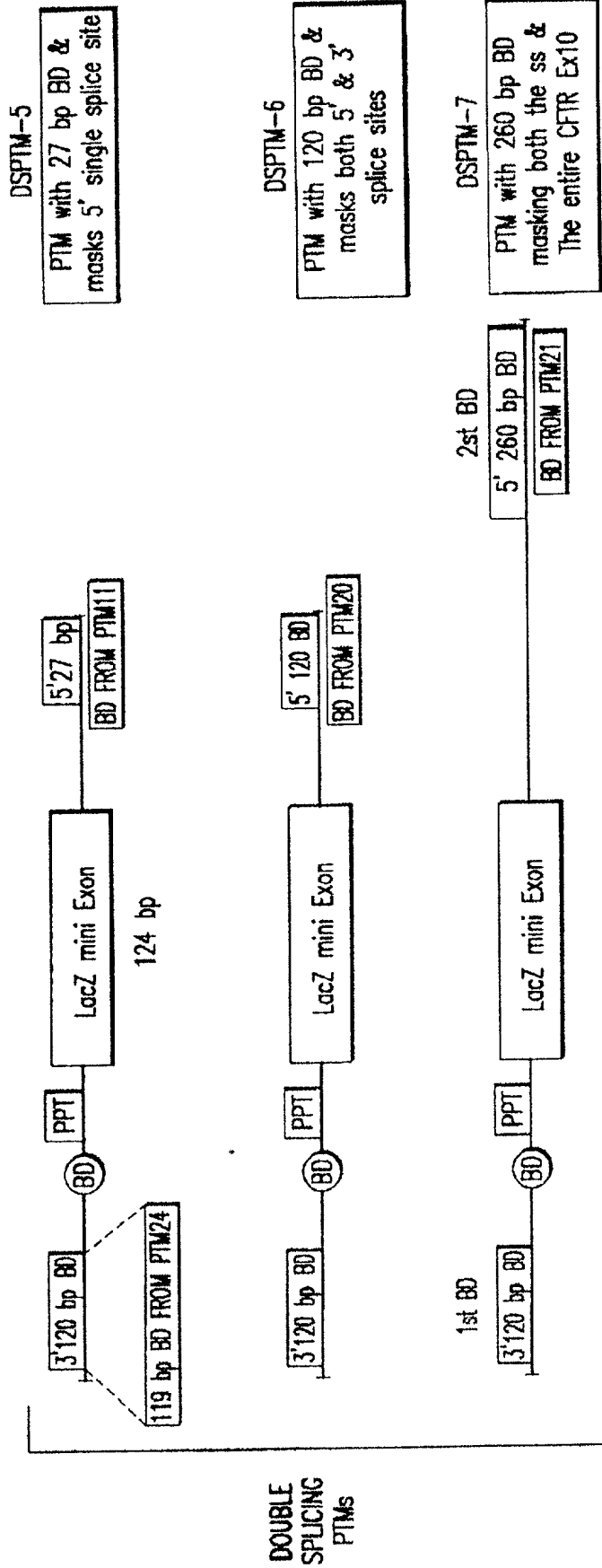
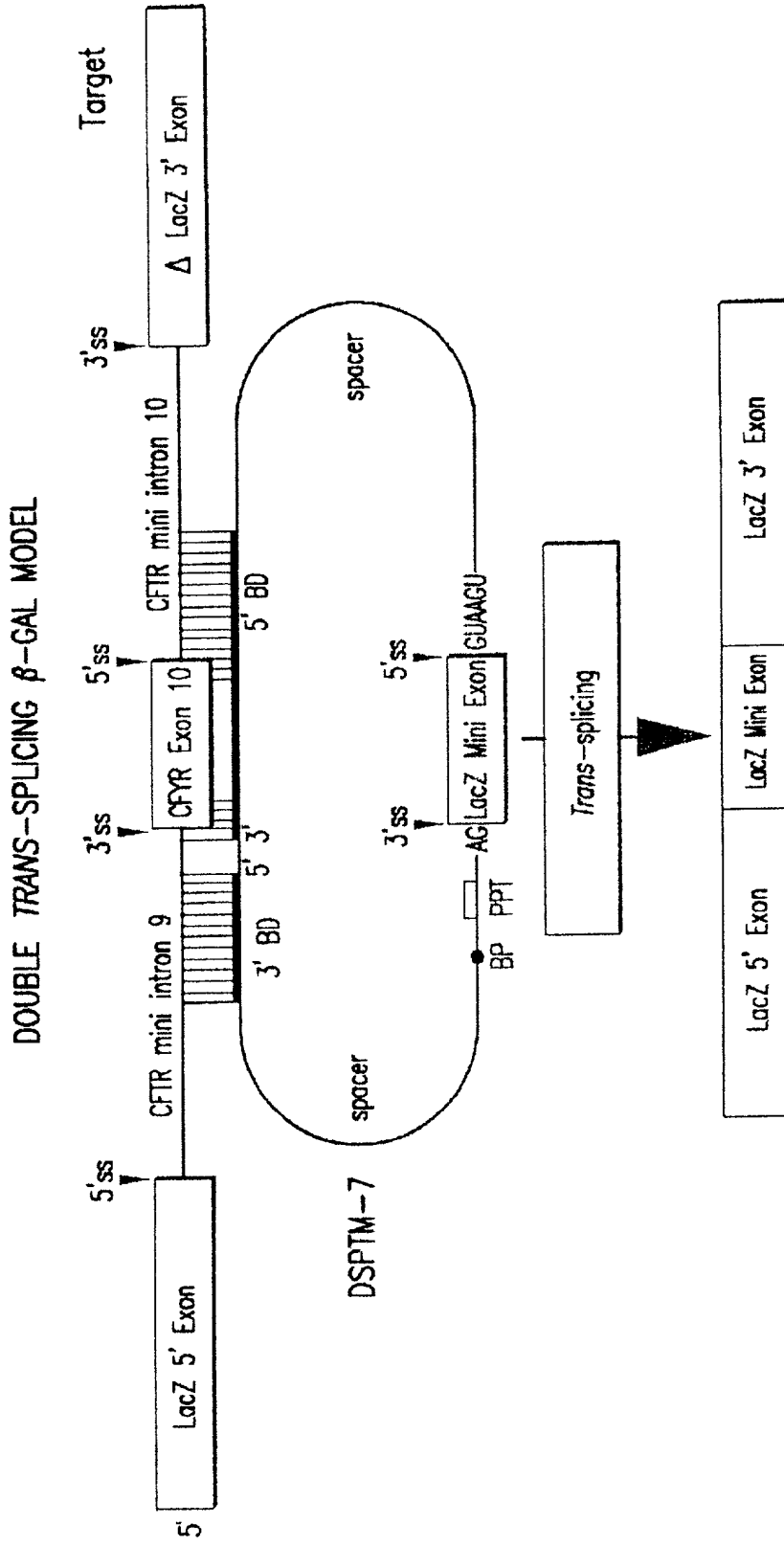
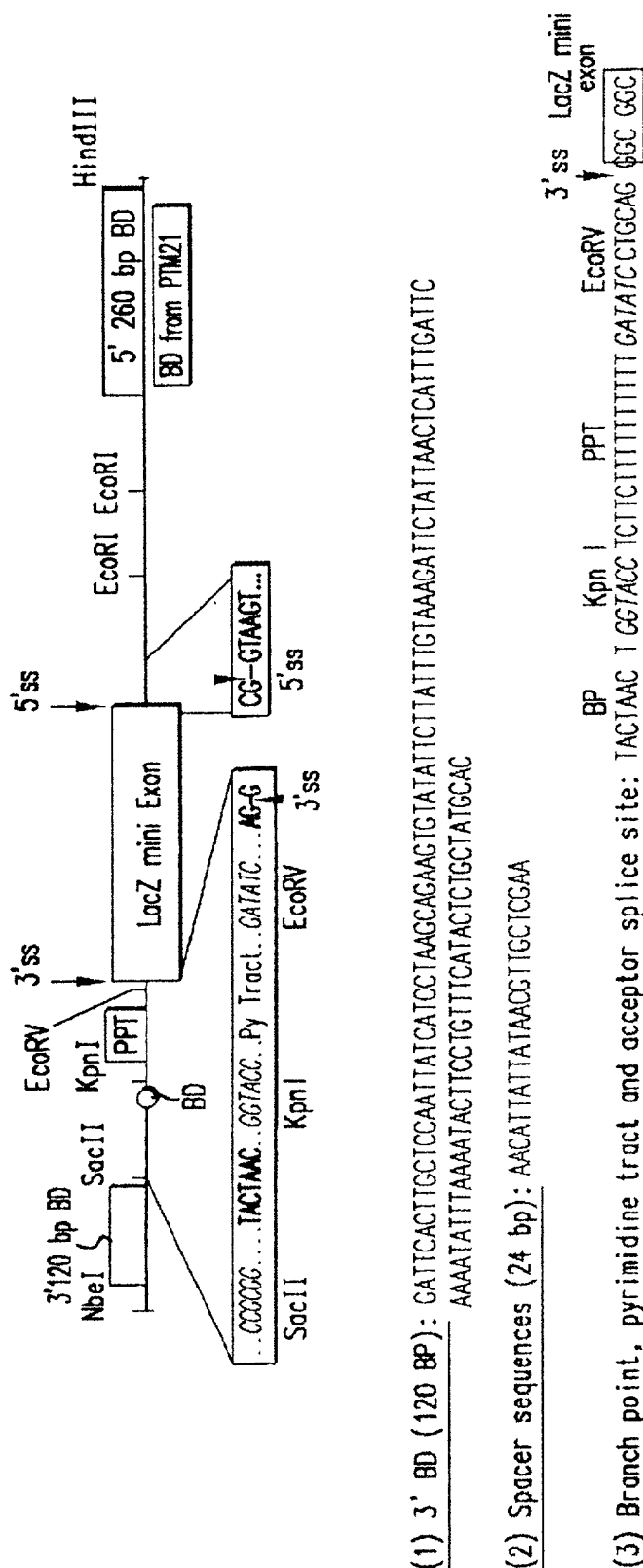


FIG.19



Repaired LacZ mRNA

**FIG.20**



(1) 3' BD (120 BP): GATTCACITGCTCCAAITATCATCTTAAGCAGAAGTGTATATCTTATTGTGAAGATCTATTAACATCATTTGATTC  
AAAAATTTAAAAATACTTCCTGTTTCATACCTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC TGG/ACC TCCTCTTTTTTTTTT GATATCCTGCAG GGC GGC

(4) 5' donor site and 2<sup>nd</sup> spacer sequence: TGA ACCGCTAACT GTTATCACC GATATGTGCTAACCTGATTCGGGCTTCGATACG

LacZ mini 5' ss  
exon

CTAAGATCCACCGG

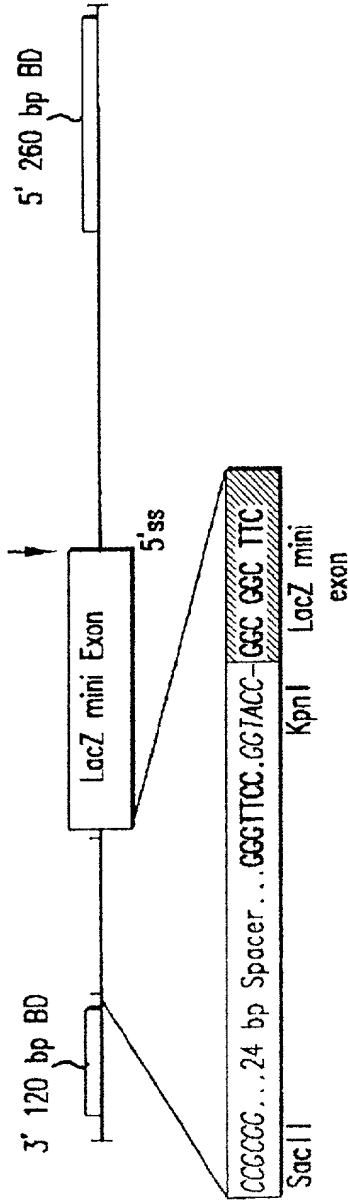
**(5) 5' BD (260 BP):** TCAAAAAGTTTCACATAATTTCTTACCTCTCTTCTGAAATTCATGCTTTGATGAGCTTCGTATCTATATTCATCATTTGGAAACACCAATGATTTTCTTTAATGGTGGCATAATCCTGGAAACGTATACACAAATCAAATTTCTCCACTGTGCTTAA  
AAAAACCCCTCGAAATTCCTCCATTTCTCCCATATCATCATTTACAAGTGAAGCTCTGGAAATAAACCCCATCATTTATTAACTCATATCAAAATCAGGC

FIG. 21

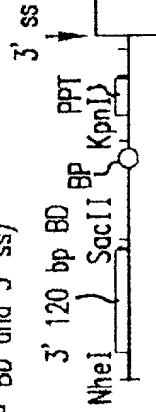
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Mutants

DSPTM8: ( $\Delta$  3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)

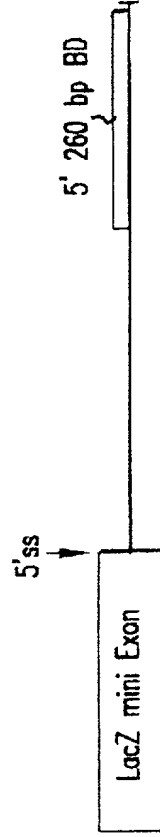


FIG.22



# ACCURACY OF DOUBLE TRANS-SPICING REACTION

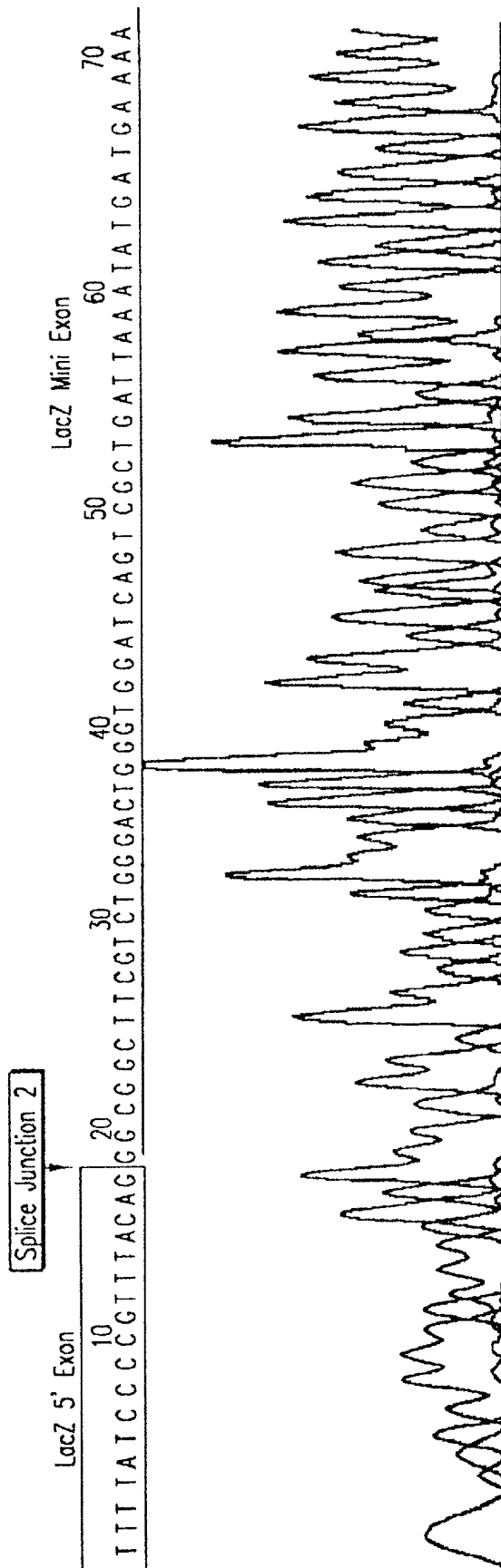


FIG.23A

# ACCURACY OF DOUBLE TRANS-SPLICING REACTION

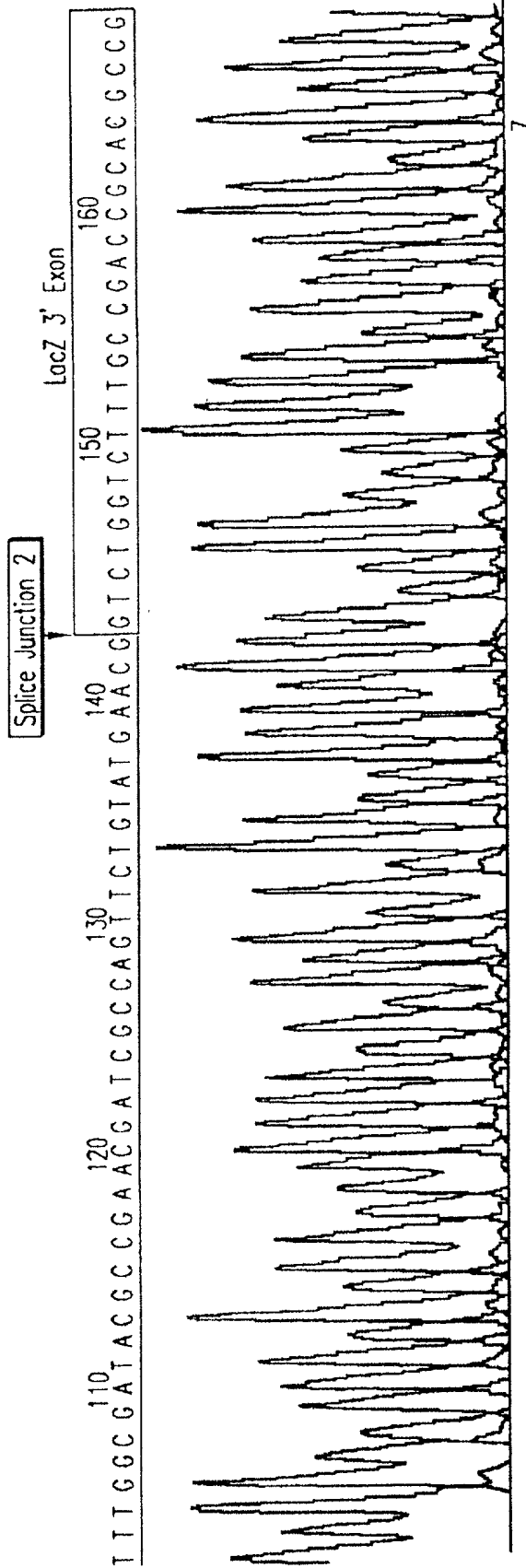


FIG.23B

**Double Trans-splicing Produces Full-length Protein**

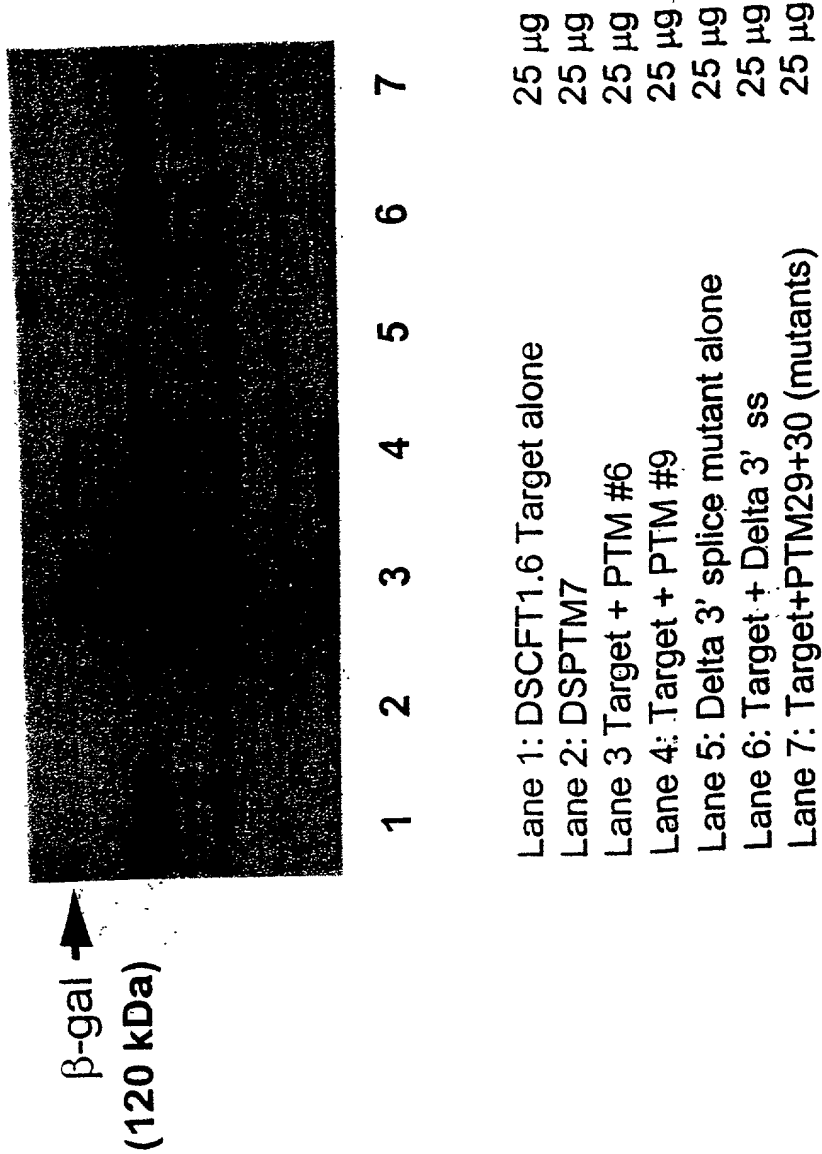


Figure 24

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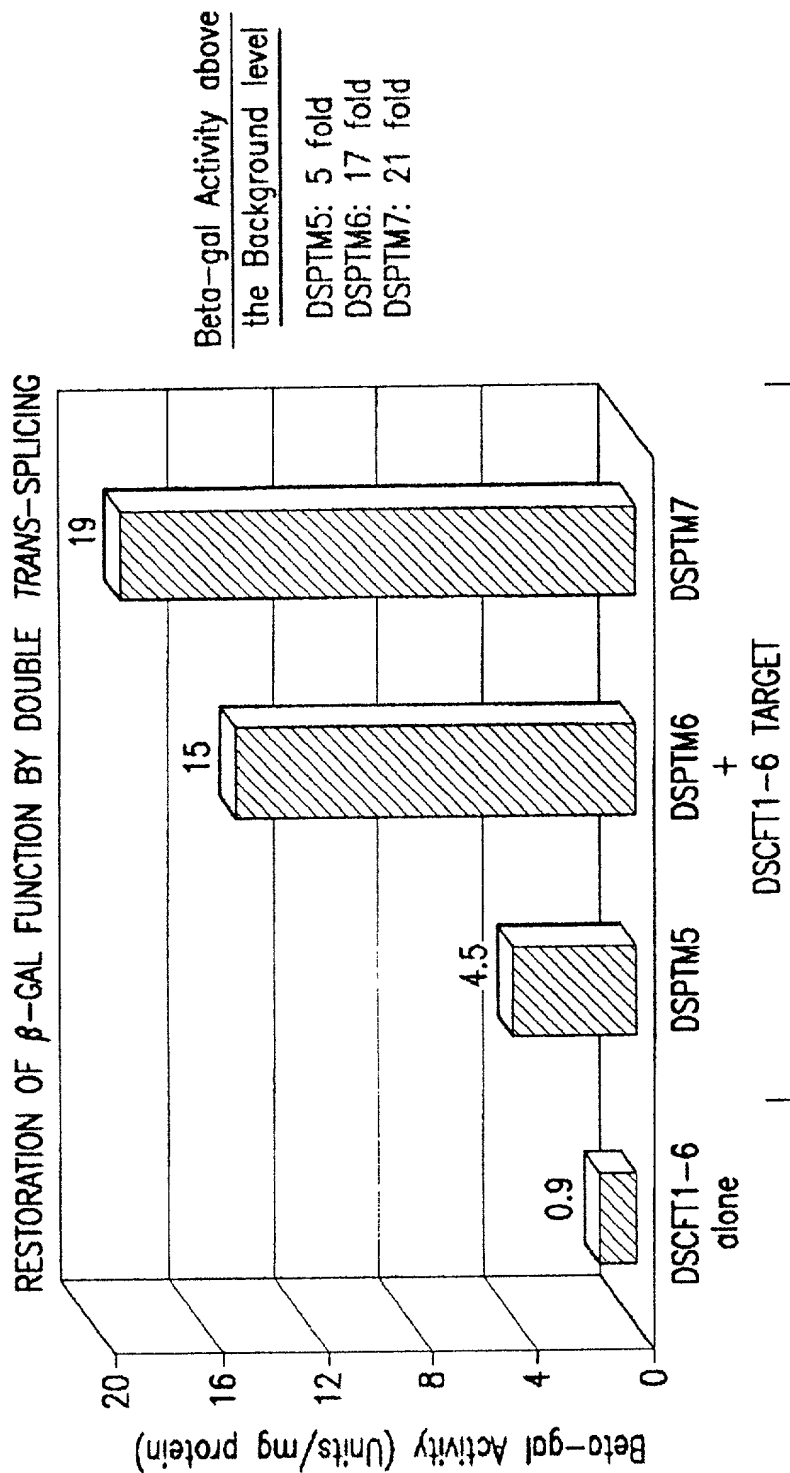


FIG.25

# RESTORATION OF $\beta$ -GAL ACTIVITY IS DUE TO DOUBLE RNA TRANS-SPLICING EVENTS

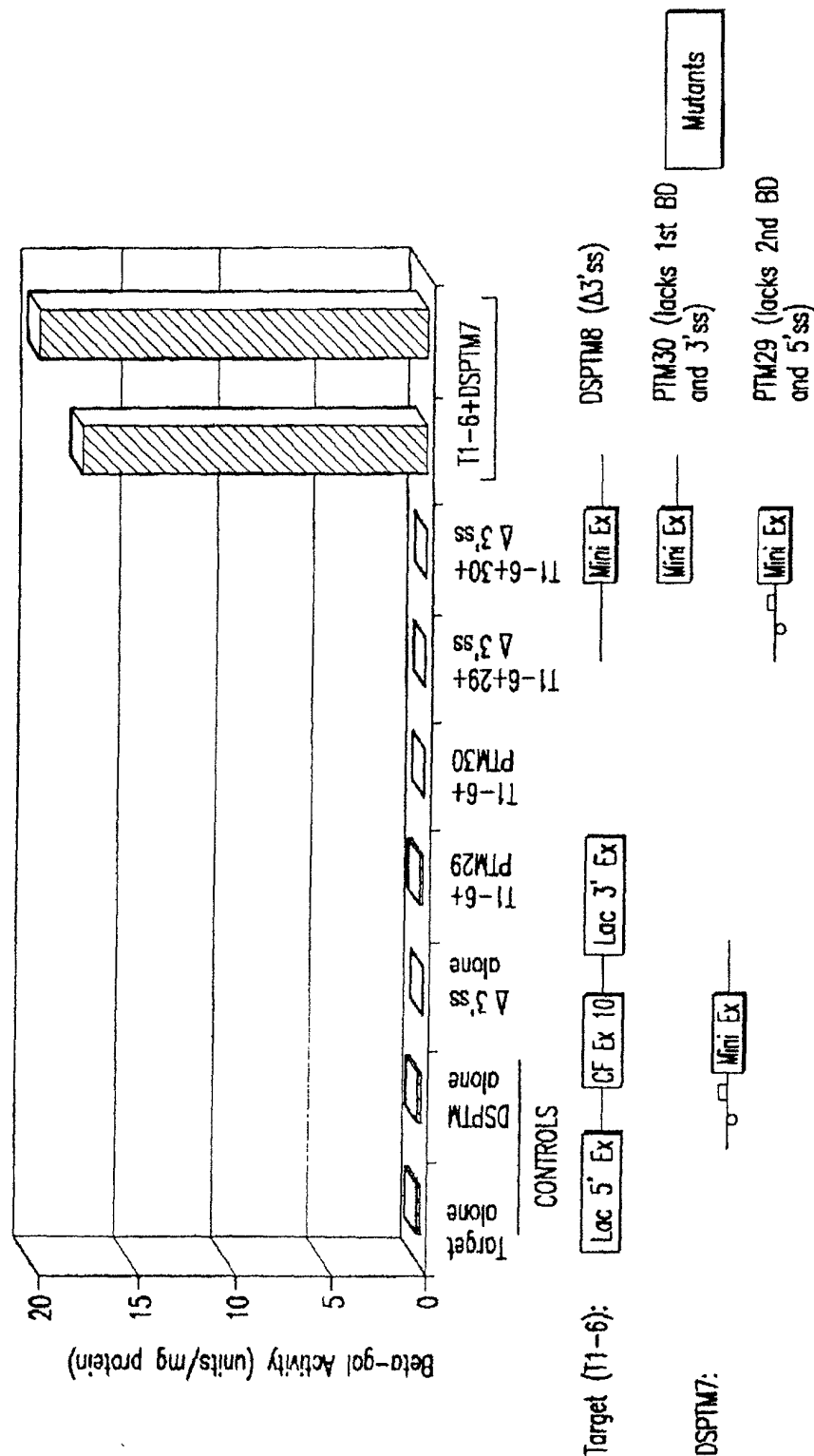


FIG.26

The current level of beta-gal activity due to double trans-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

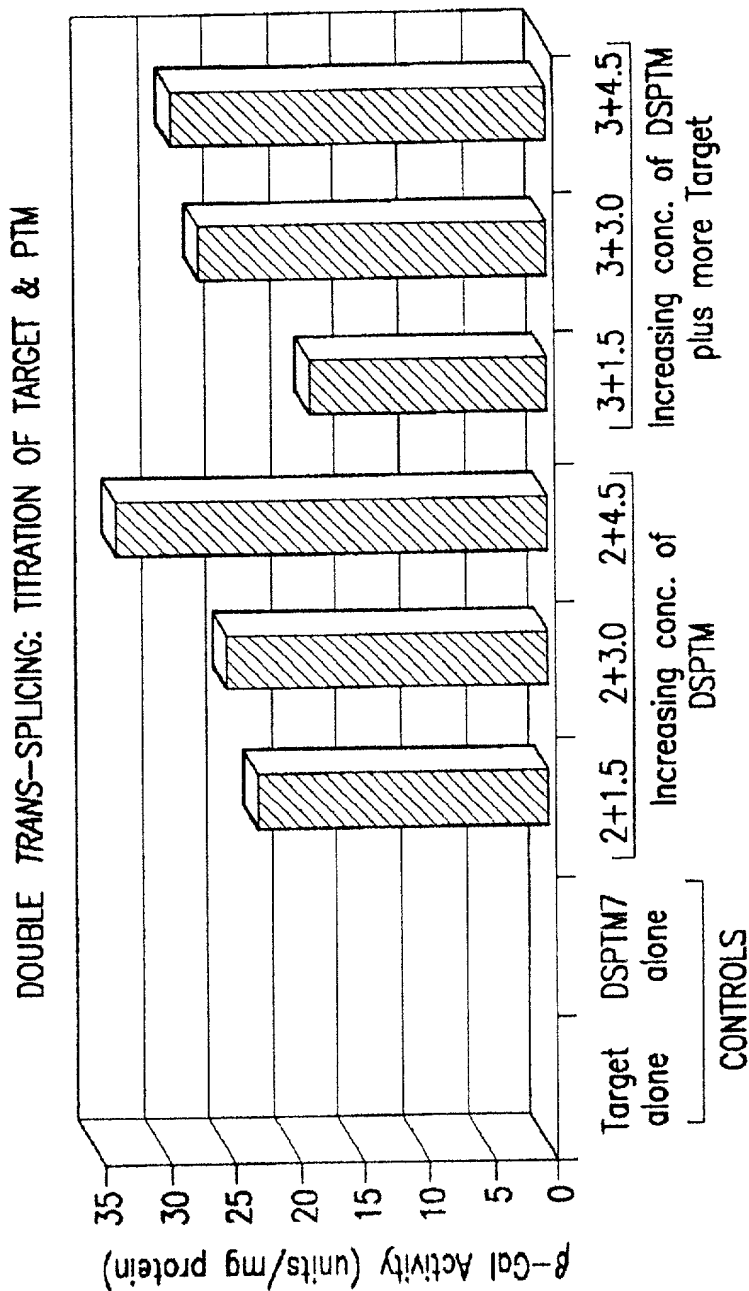


FIG.27

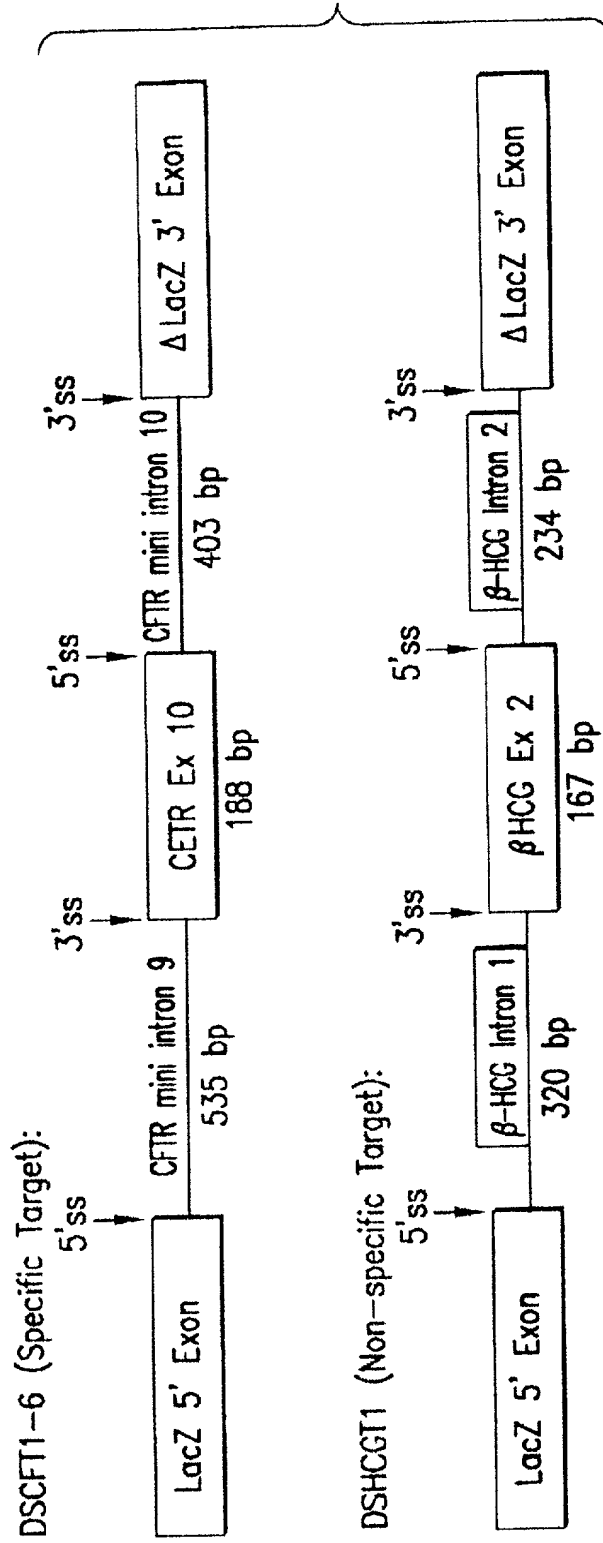


FIG.28

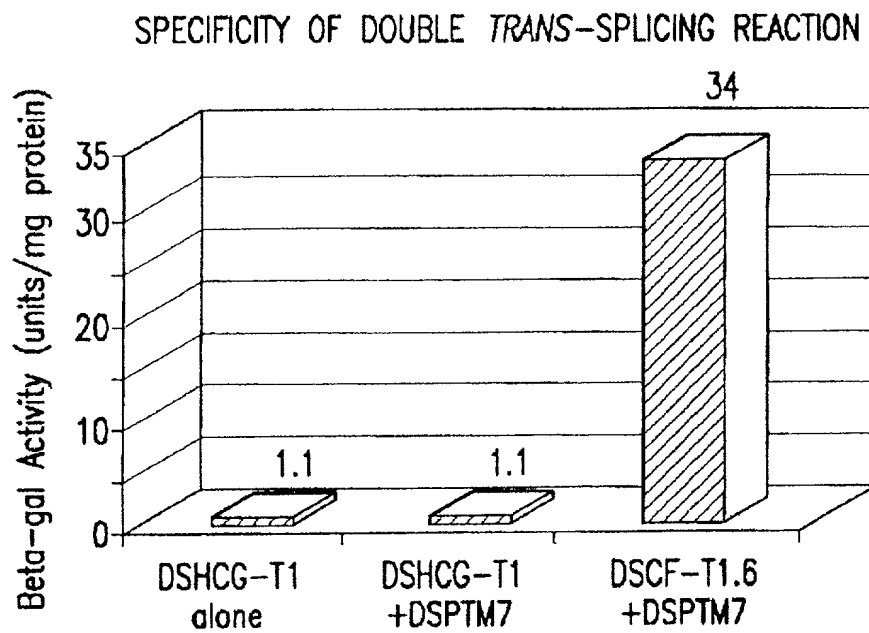


FIG.29



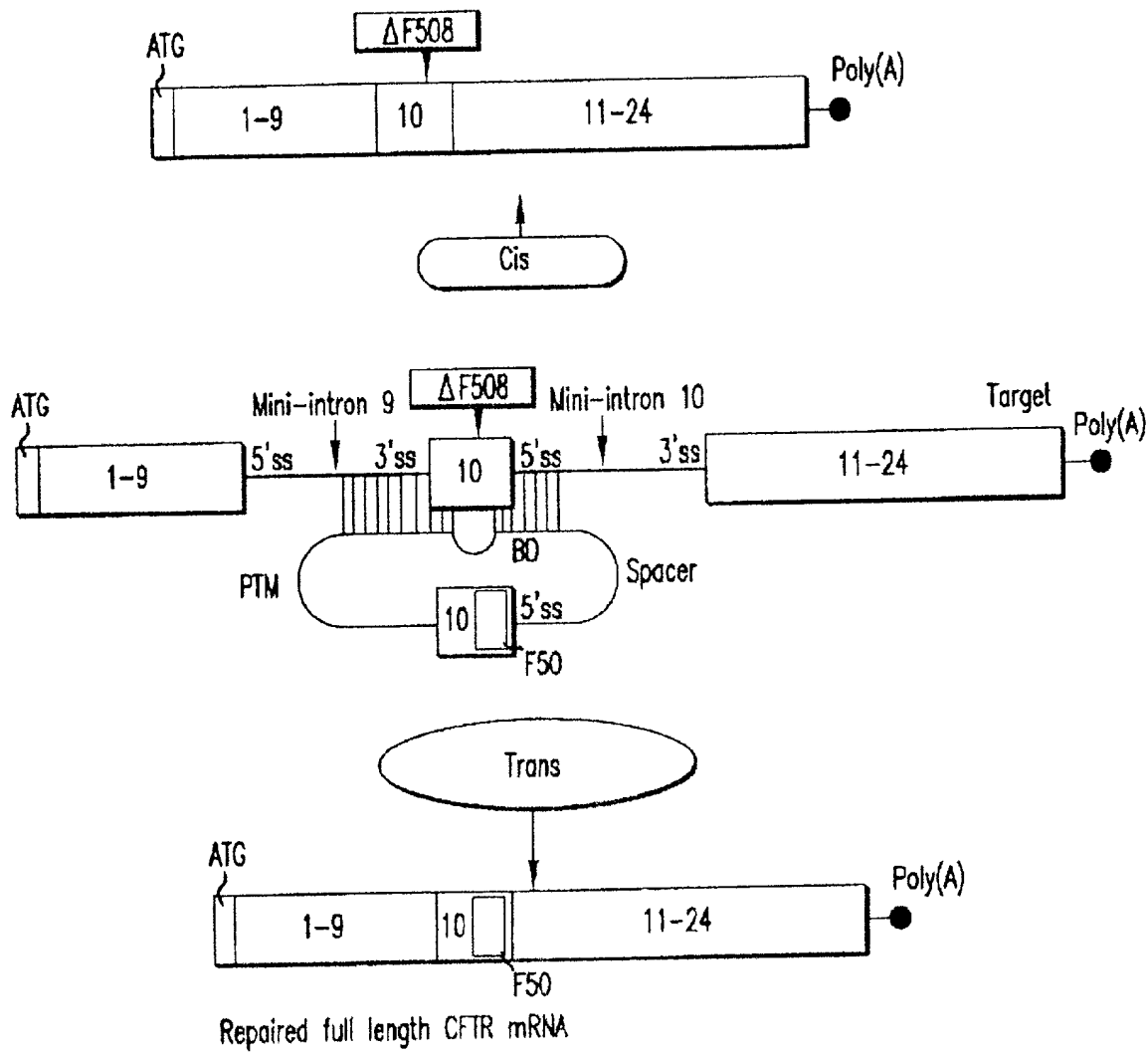
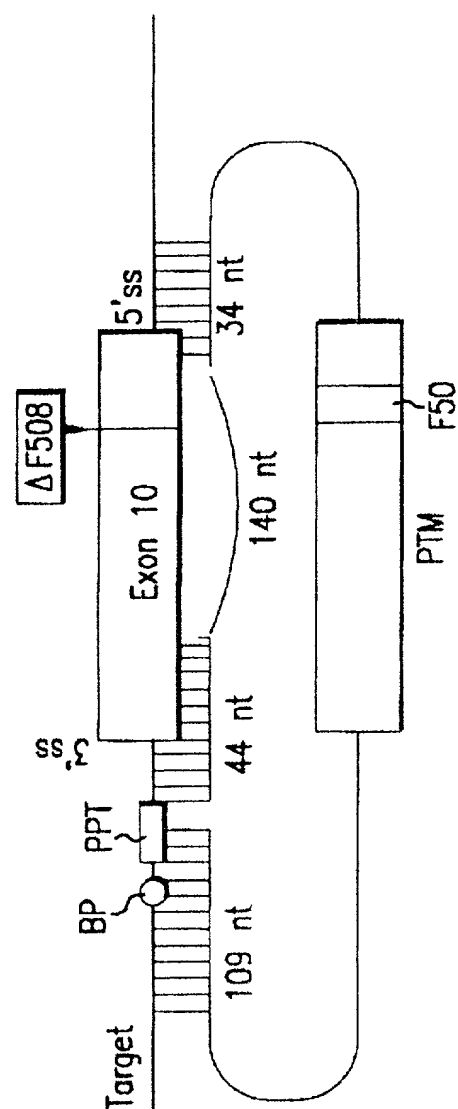


FIG.30

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PTM with a long binding domain masking  
two splice sites and part of exon 10  
in a mini-gene target



ACGAGCTTGCTCATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCGG  
GCGCATCAGCTTTTCAGGCCAATTCAGTTCGATCATGCCCGGTACCATCAAGGAGAACATAT  
CTTCGGCGTCAGTACGACGAGTACCGCTATCGCTCGGTGATTAAGCCCTGTCAGTTCGAGGAG

MCU in exon 10 of PTM

88 OF 192 (46%) bases in PTM exon 10 are not complementary to  
its binding domain (bold and underlined).

FIG.31

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# Sequence of a double Trans-spliced product

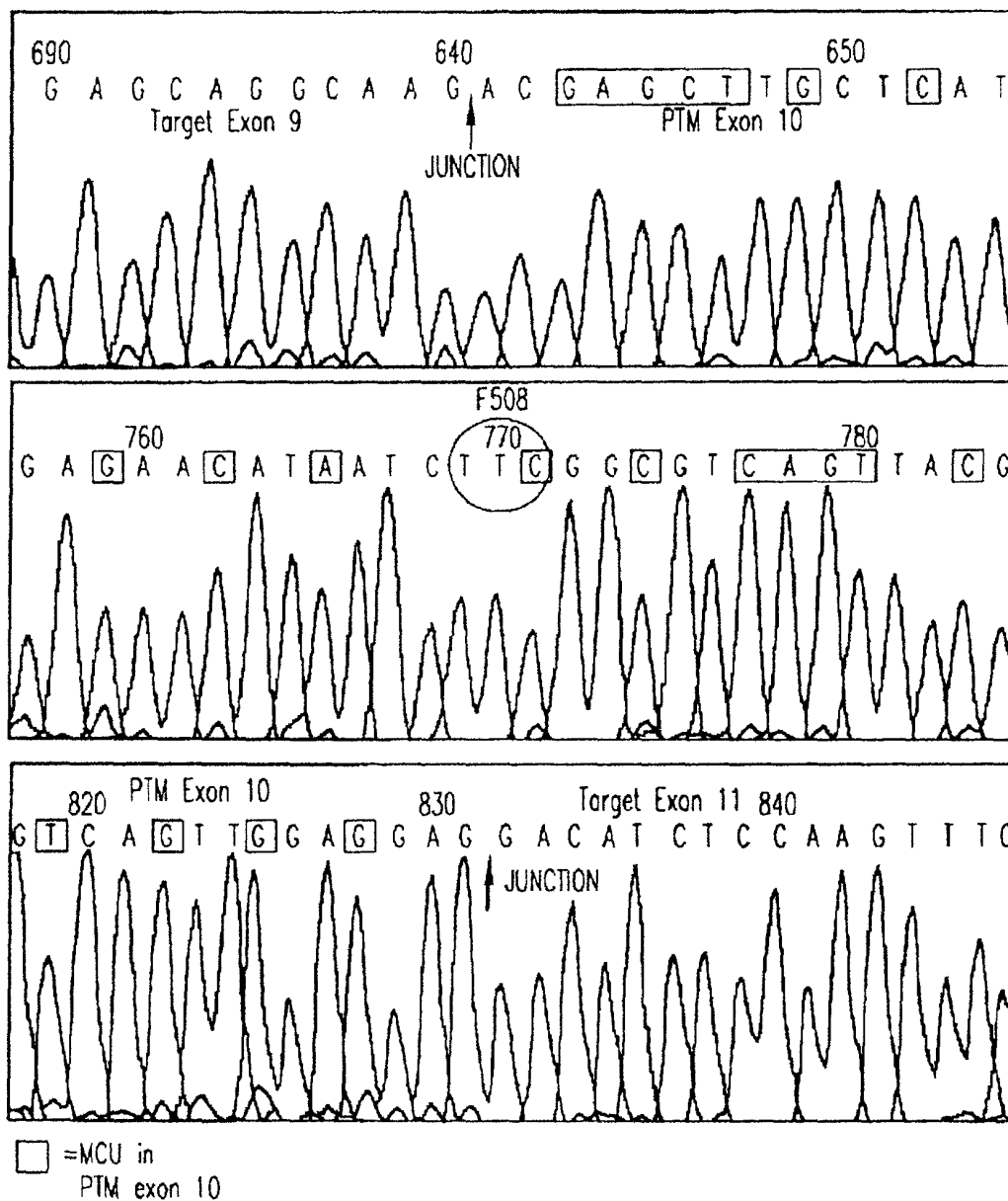


FIG.32

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CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

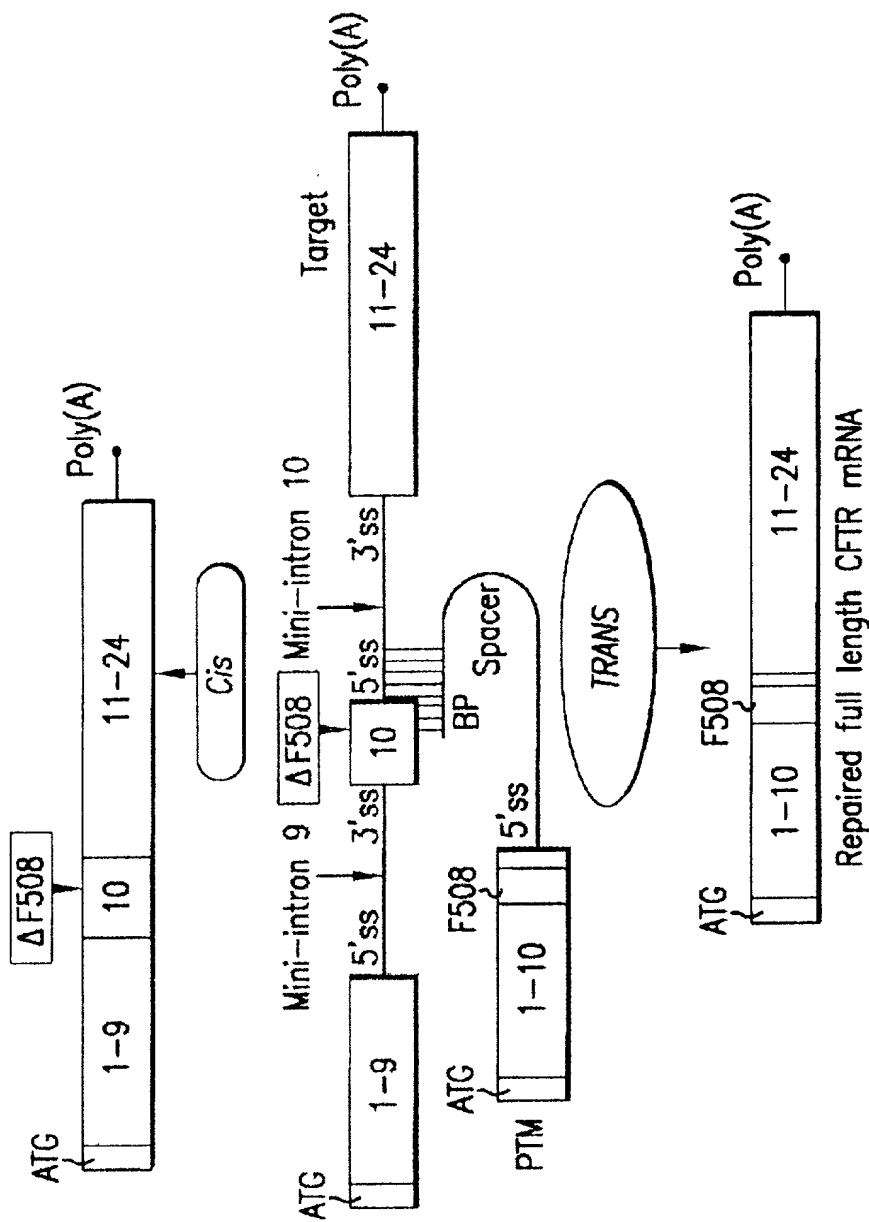


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

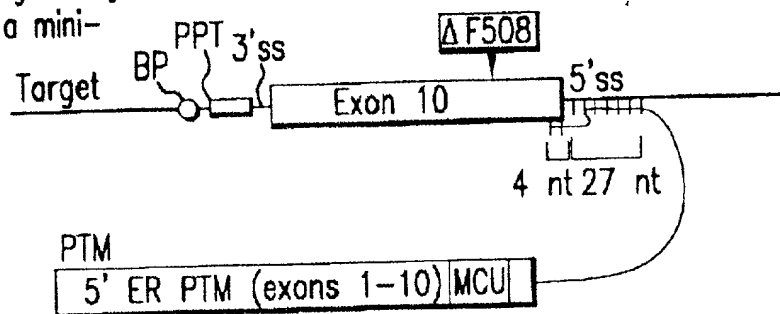


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

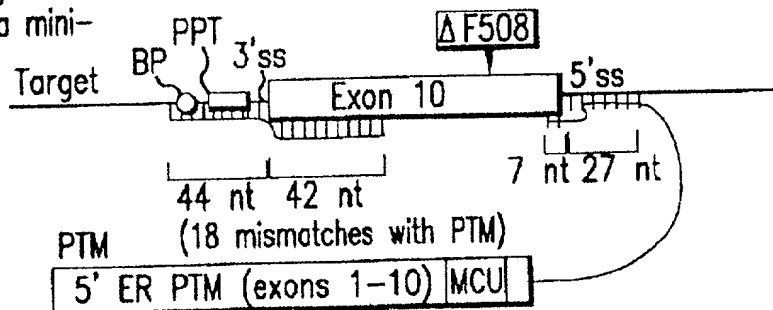


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

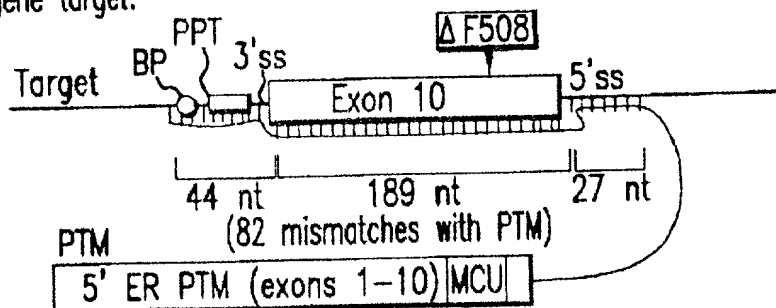
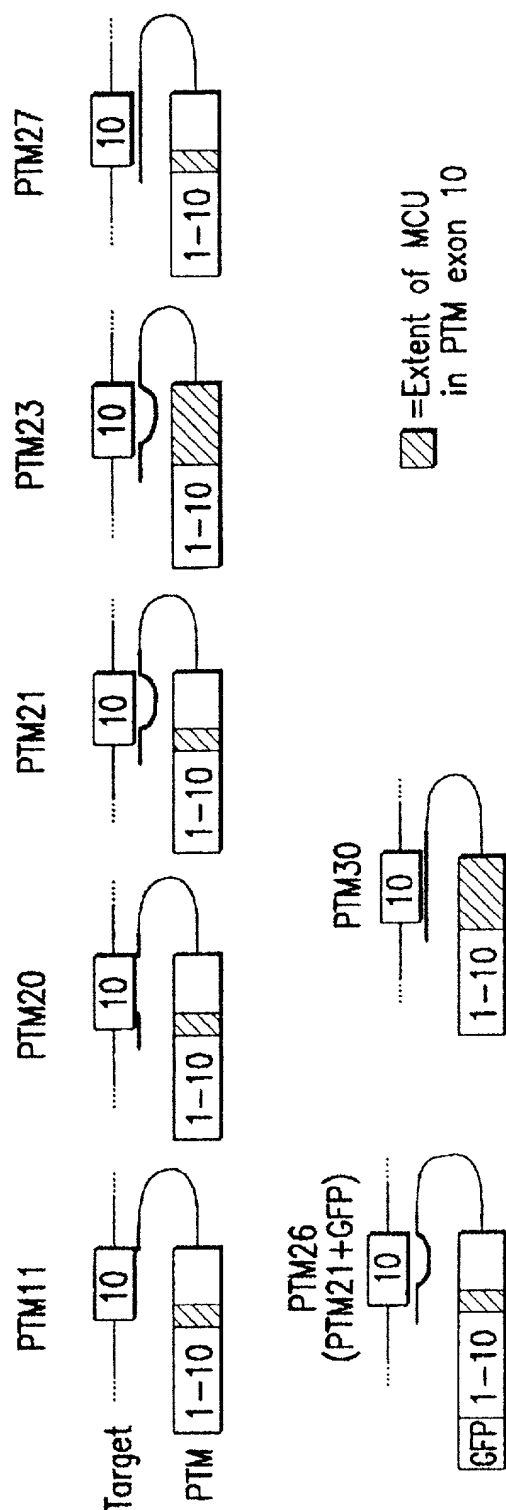


FIG.34C



MCU in exon 10 of PTM  
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGGTCATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCCG  
GCGGCATCAGCTTTTCAGGCCAATTCAGTTGGATCATGCCCGGTACCAATCAAGGAGAACATAT  
CTTCGGCGTCAGTTACGACGAGTACCGCTATCCGCTGGTGTATTAAGGCCGTGTCAGTTGGAGGAG

FIG.35

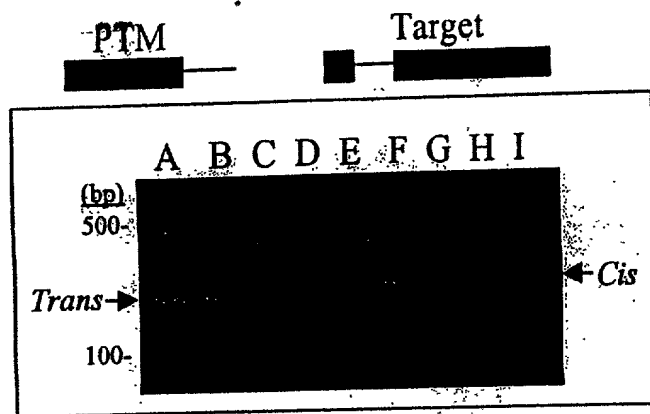


FIG. 36 A

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Cis-spliced product  
[Primers CF1+CF111]

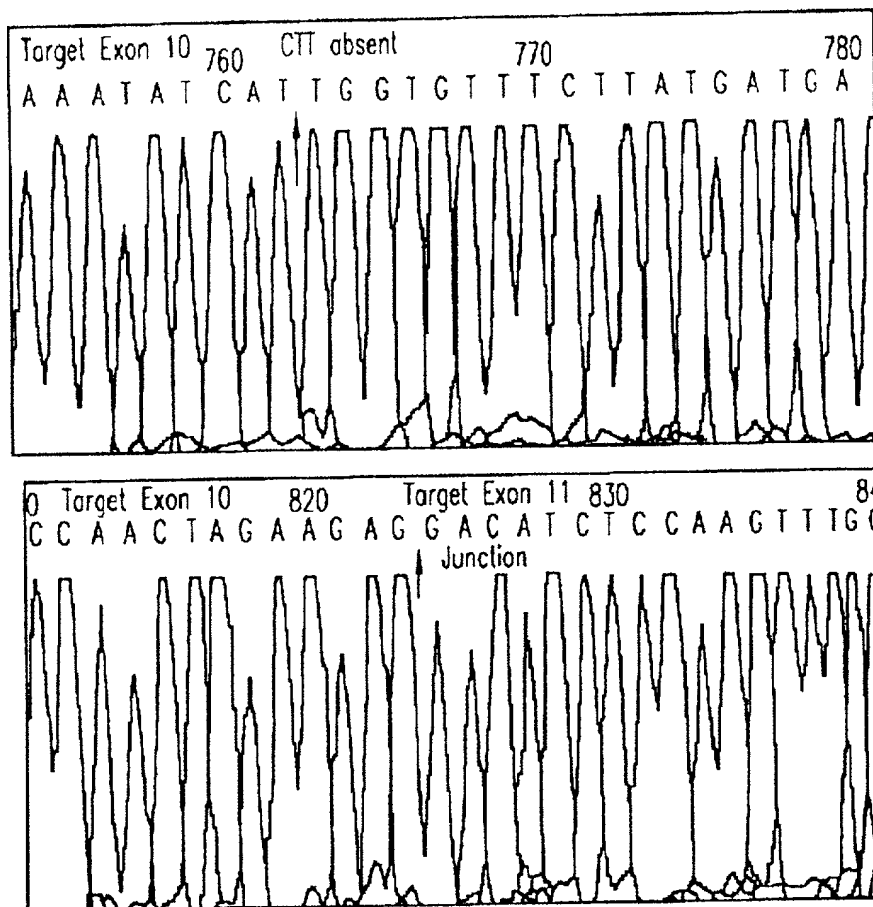


FIG.36A-1



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Trans-spliced product  
[Primers CF93+CF111]

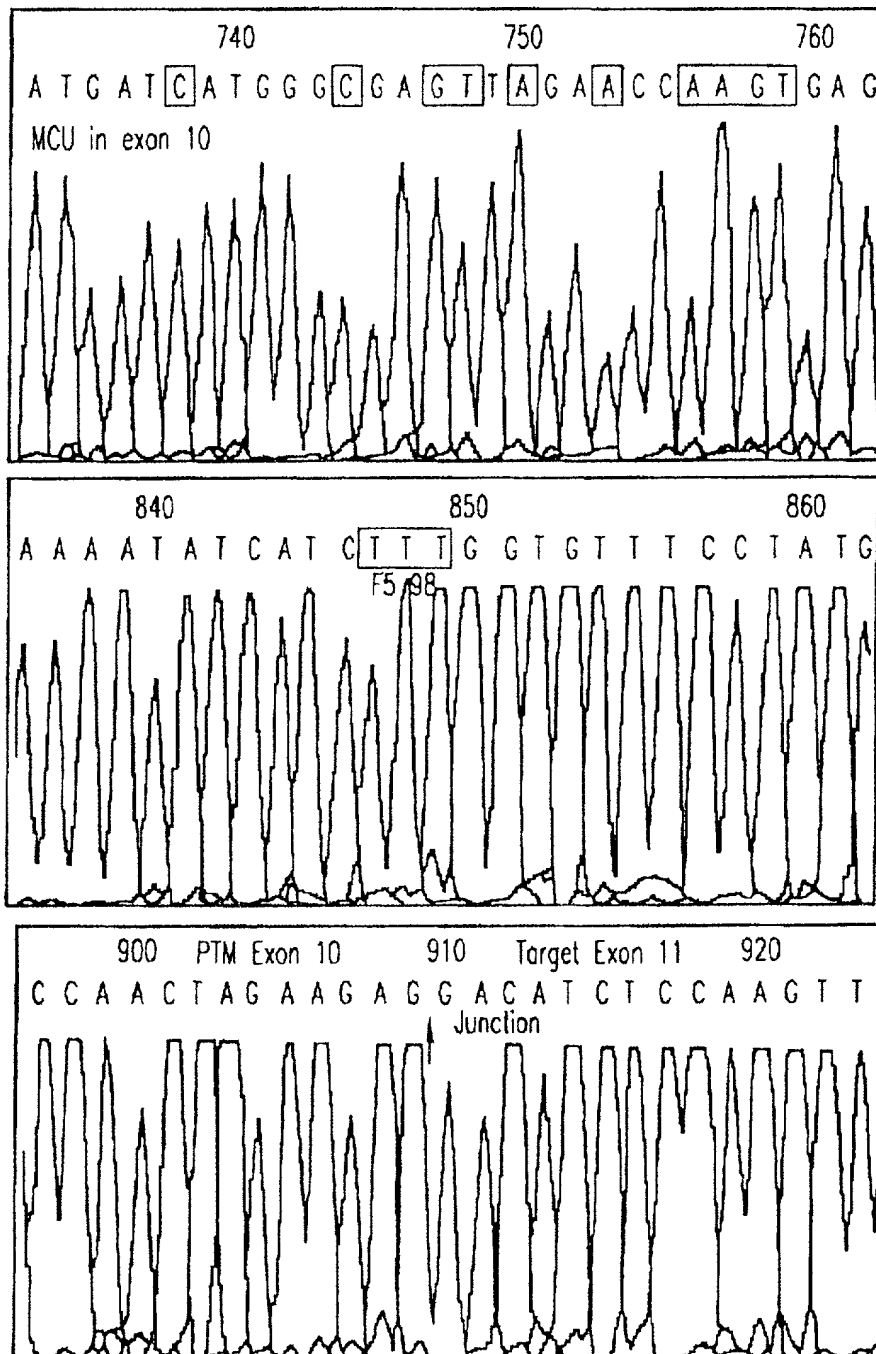


FIG.36B

204040" 25474650

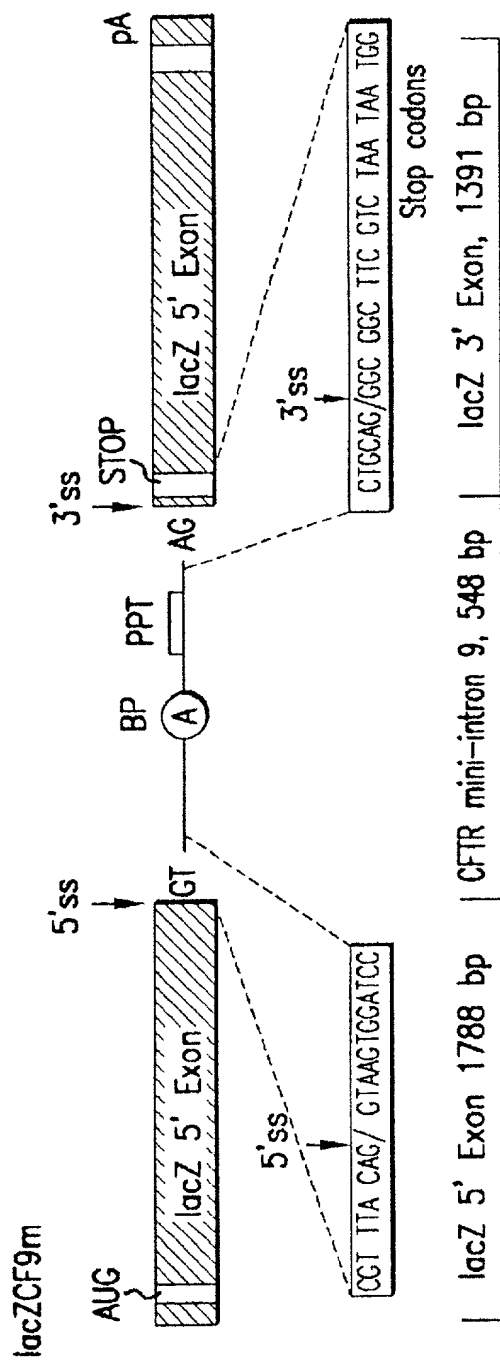


FIG.37A

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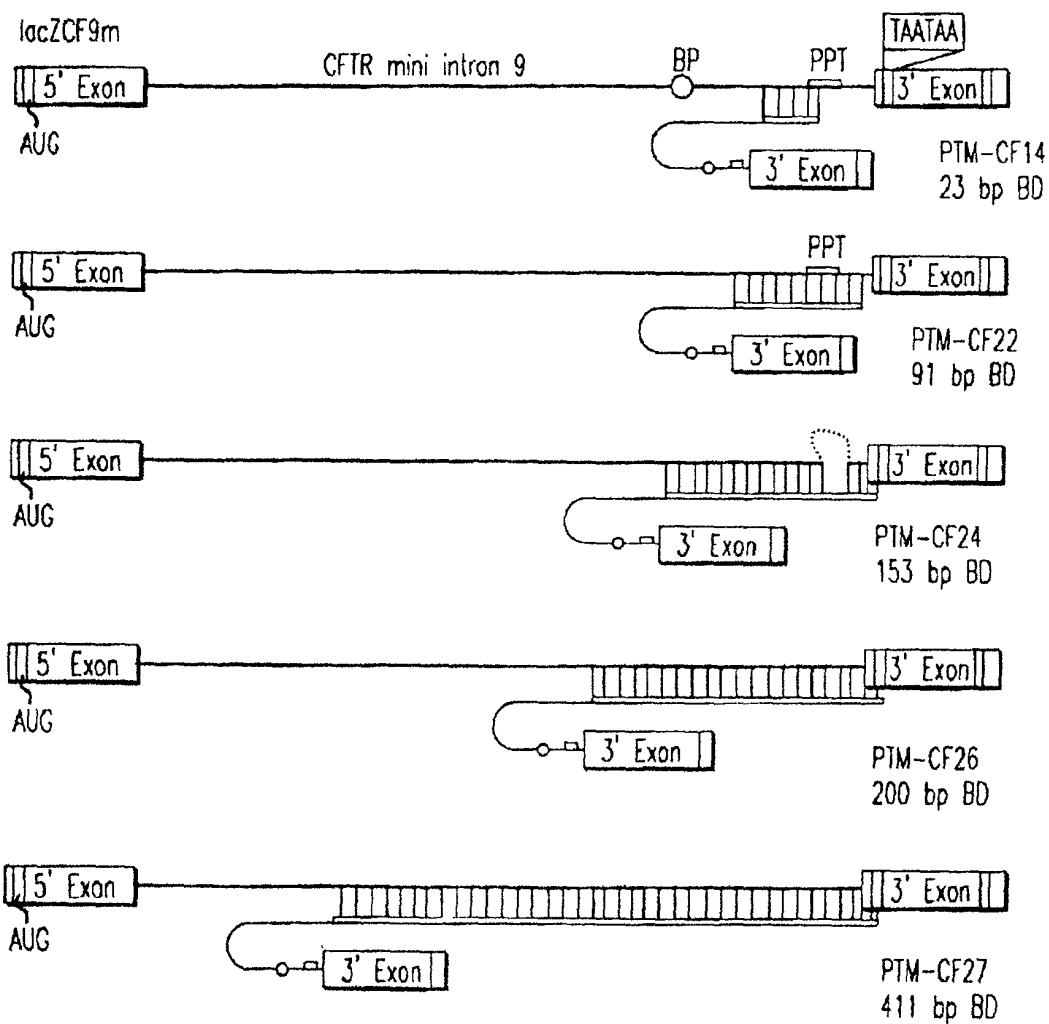
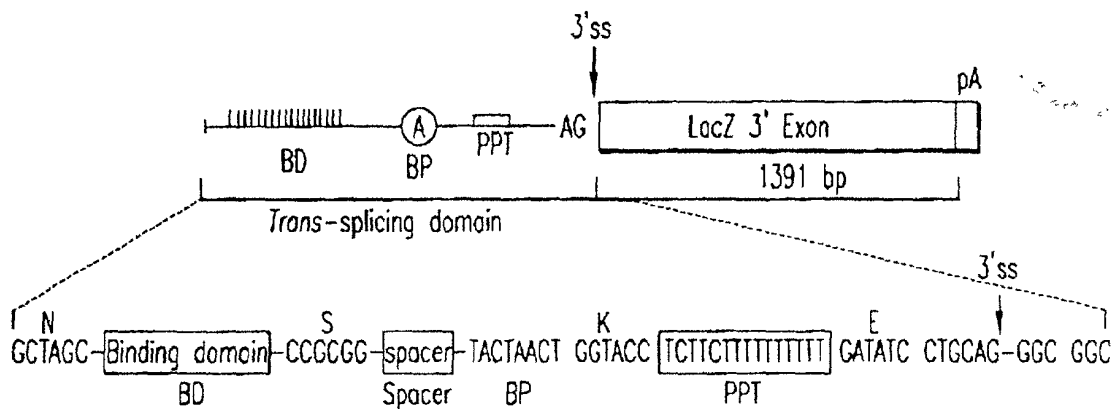


FIG.37B

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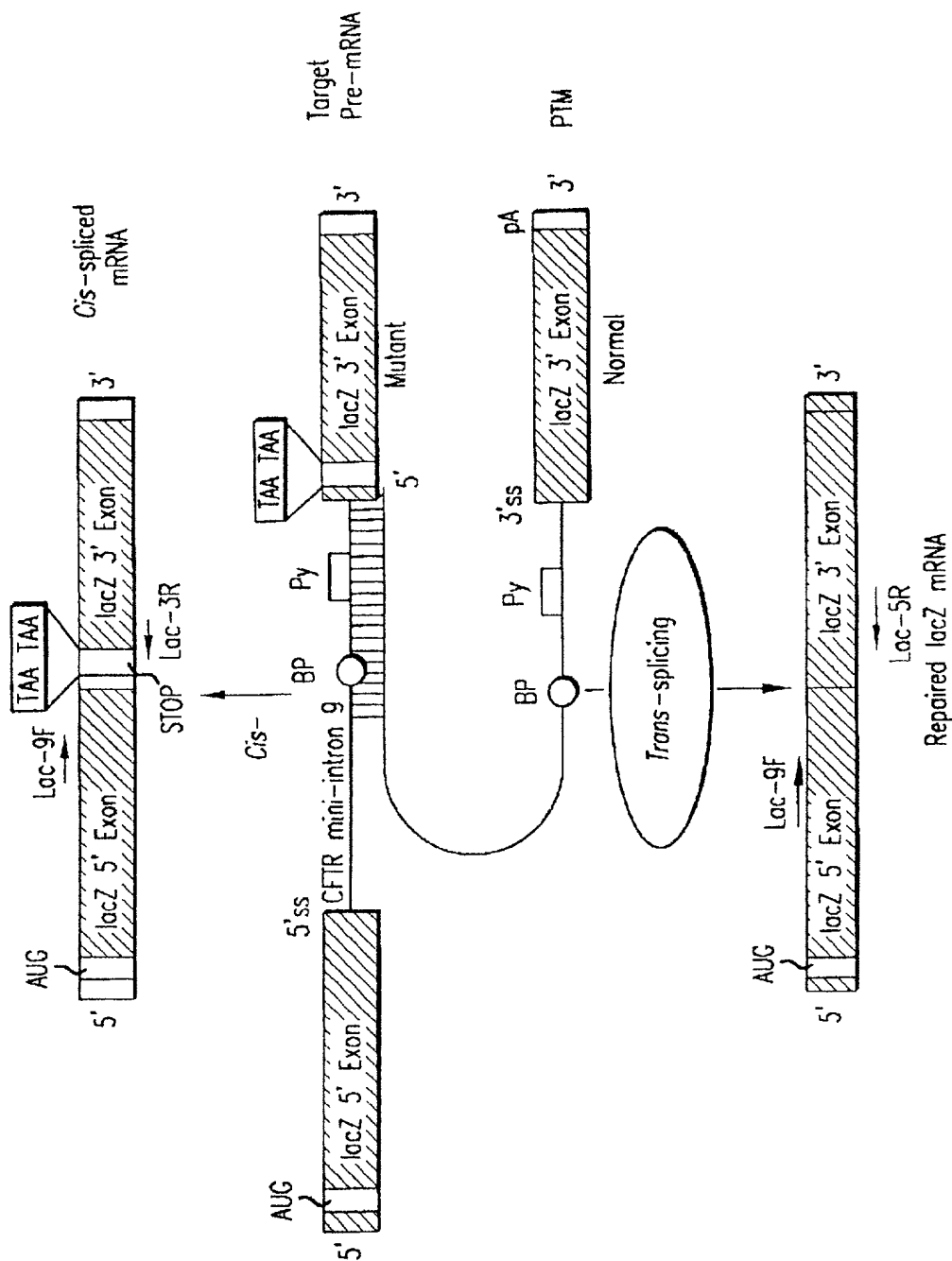


FIG.37C

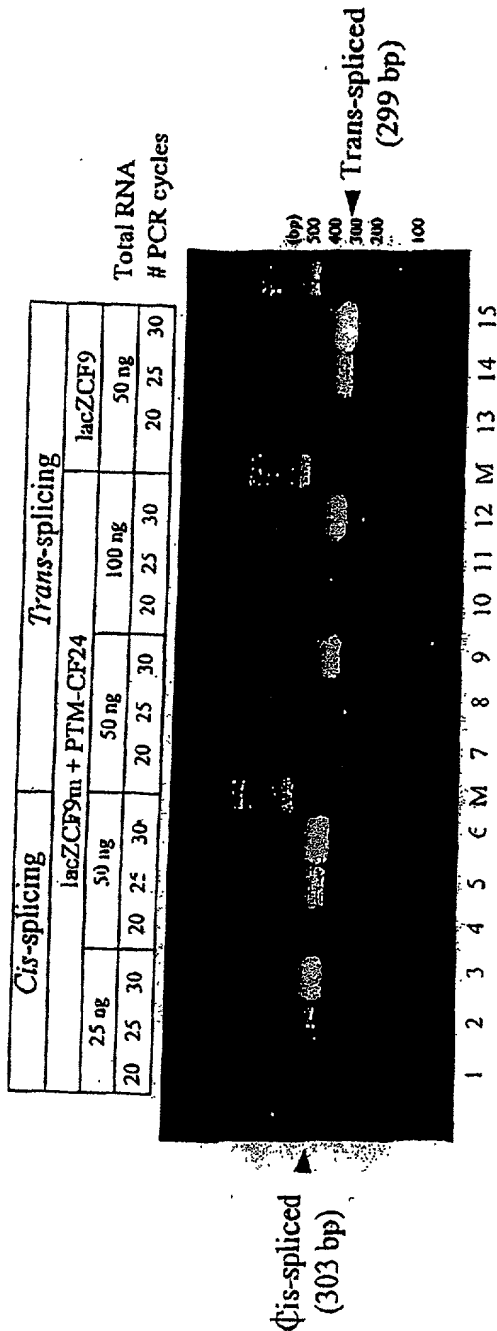
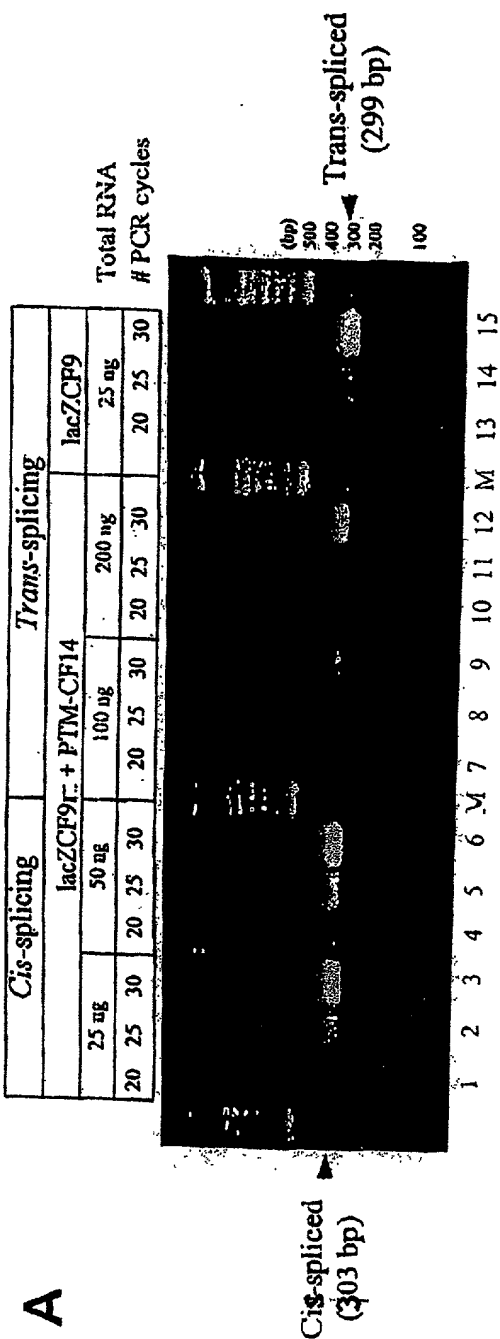


FIG. 38A

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**B**

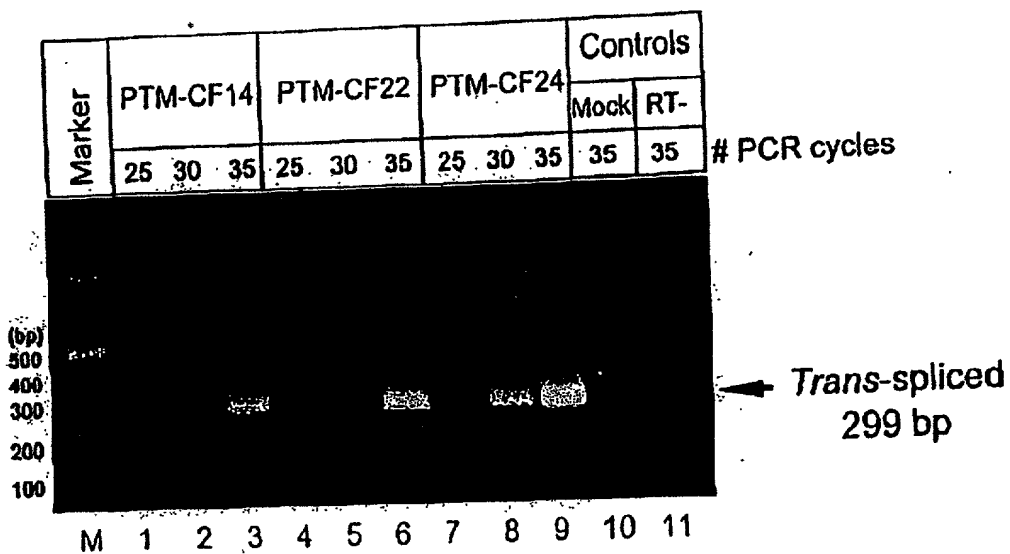


FIG. 38B

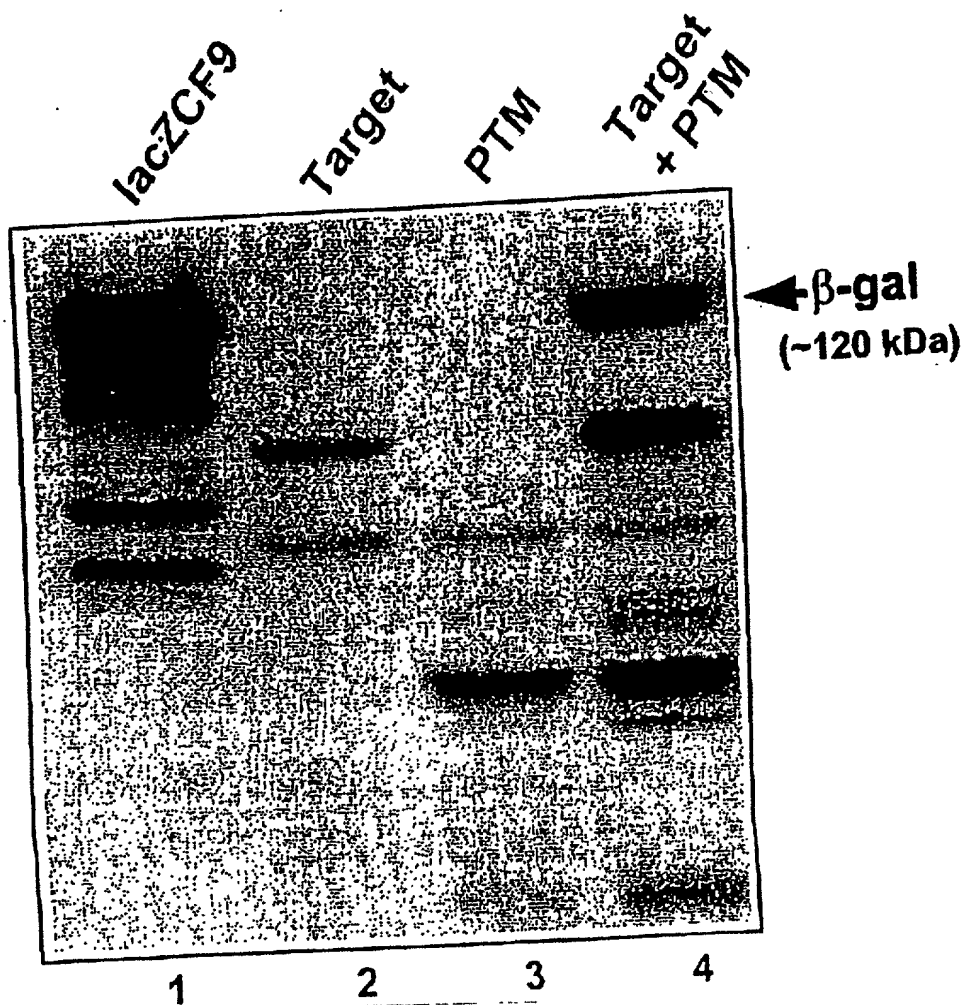


FIG. 39

204040" 2644660

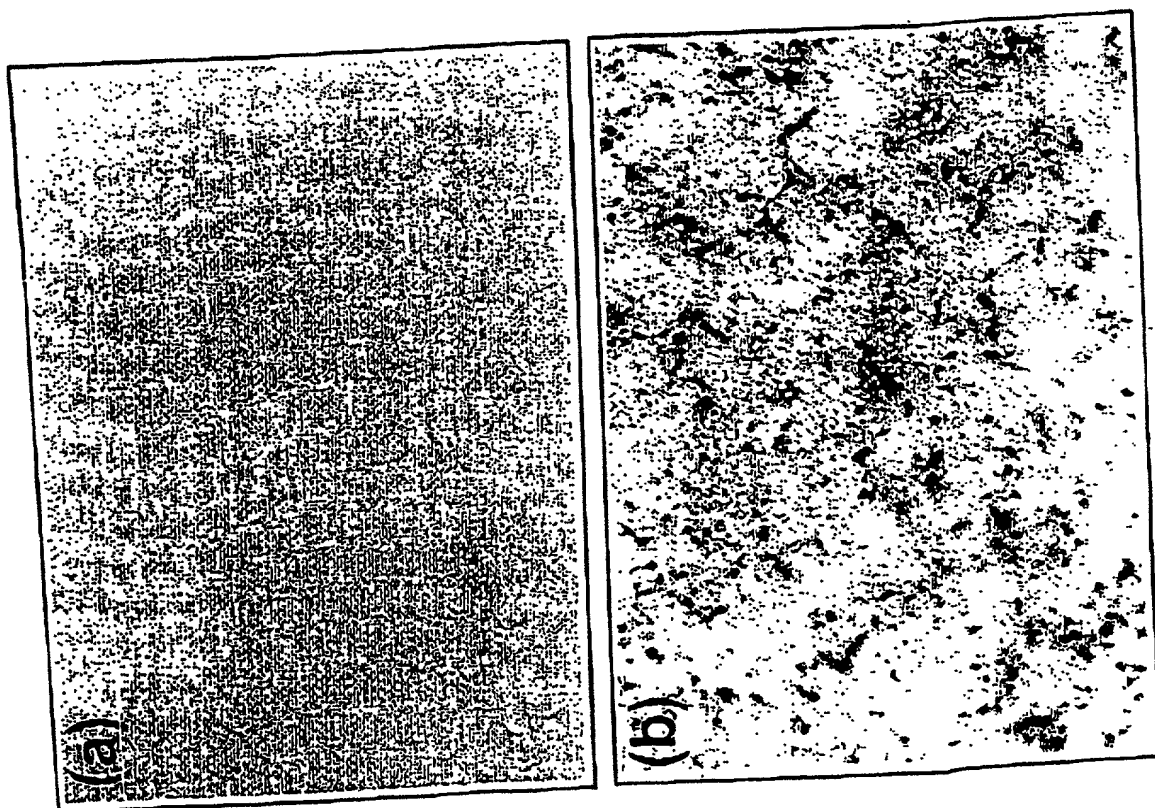


FIG. 40A

A



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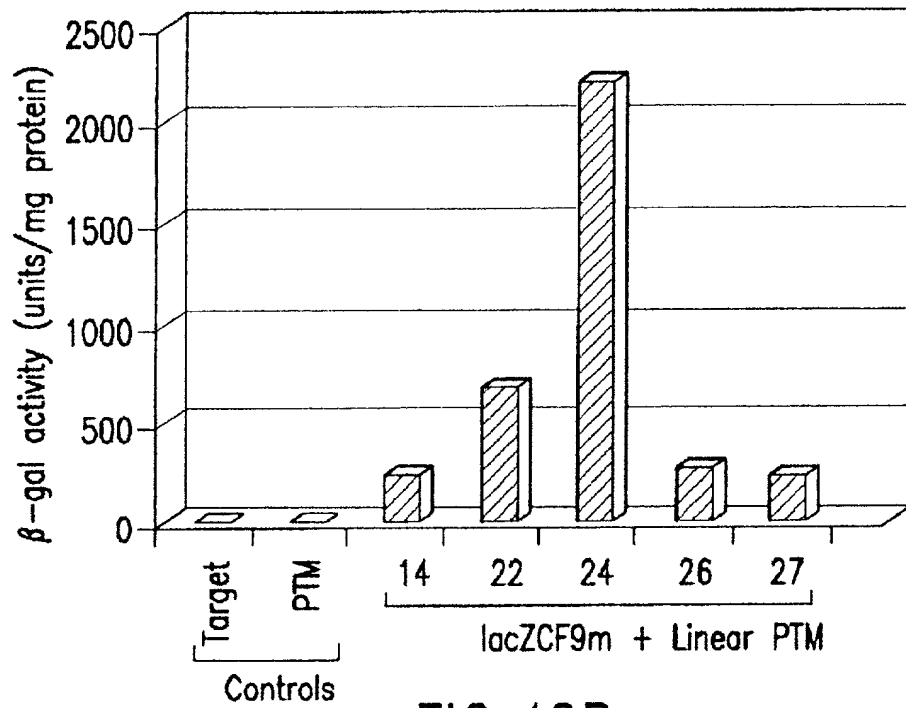


FIG.40B

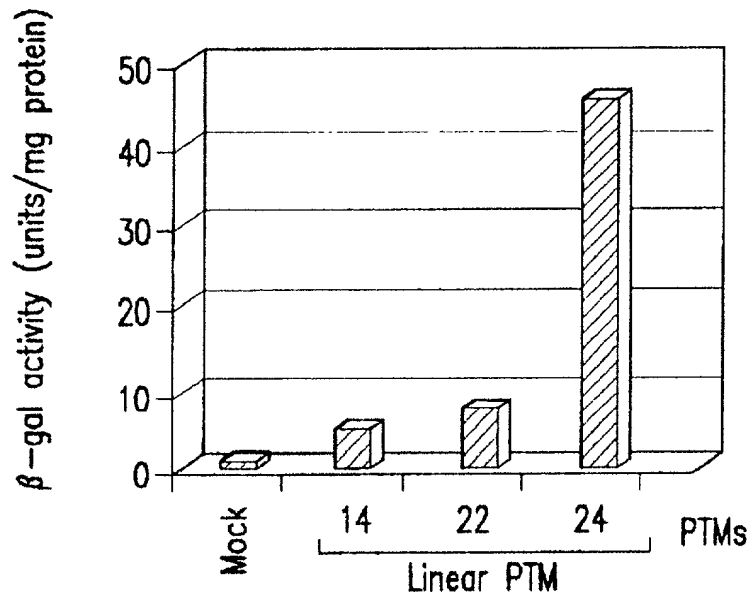


FIG.40C

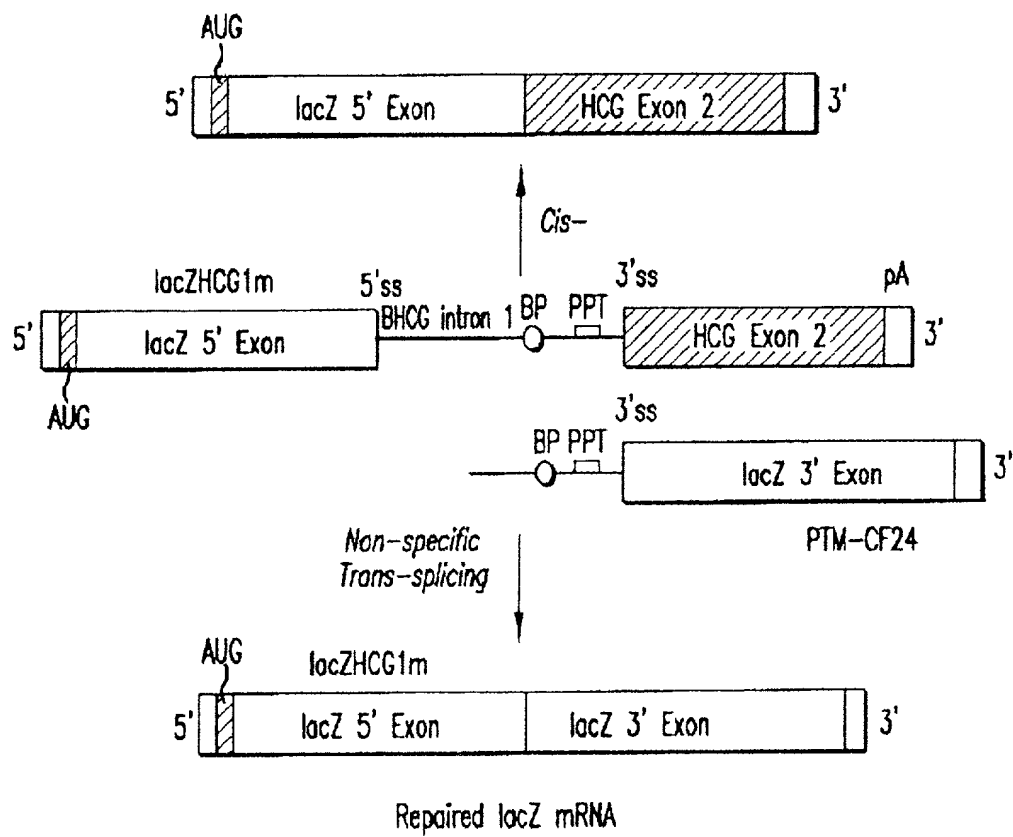


FIG.41A

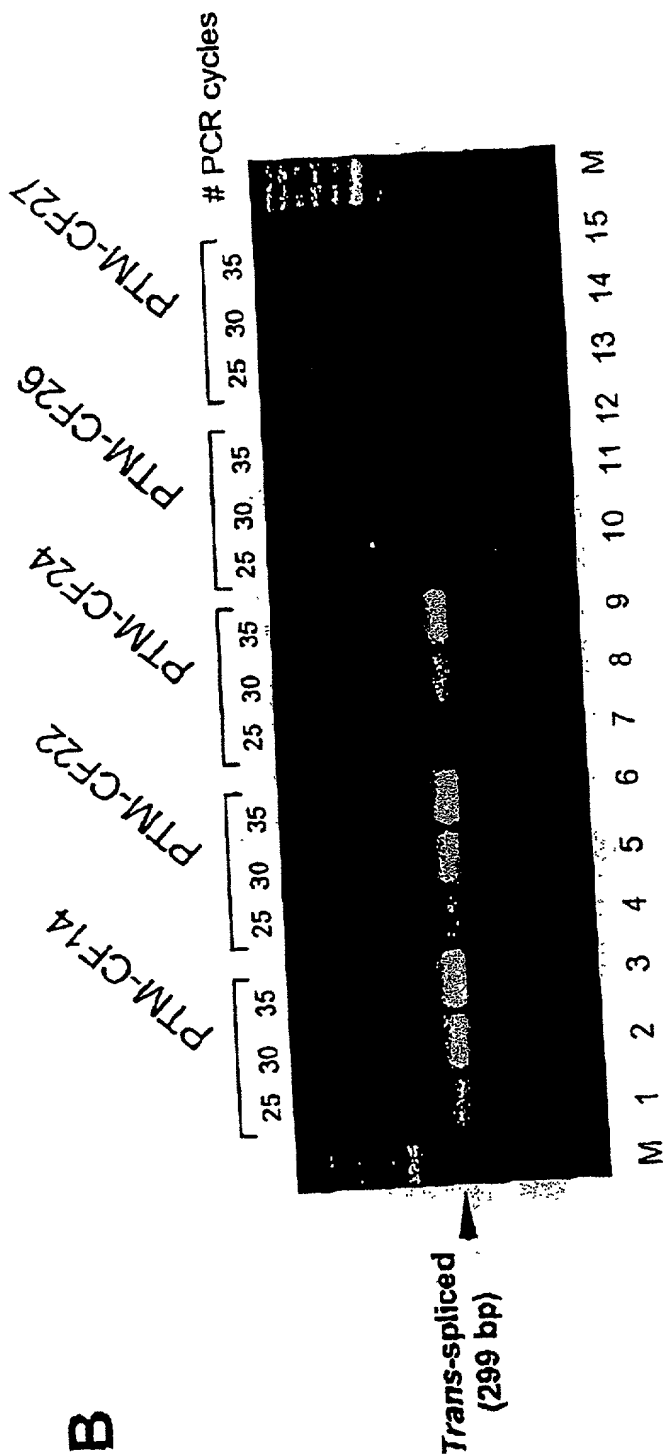


Figure 4B

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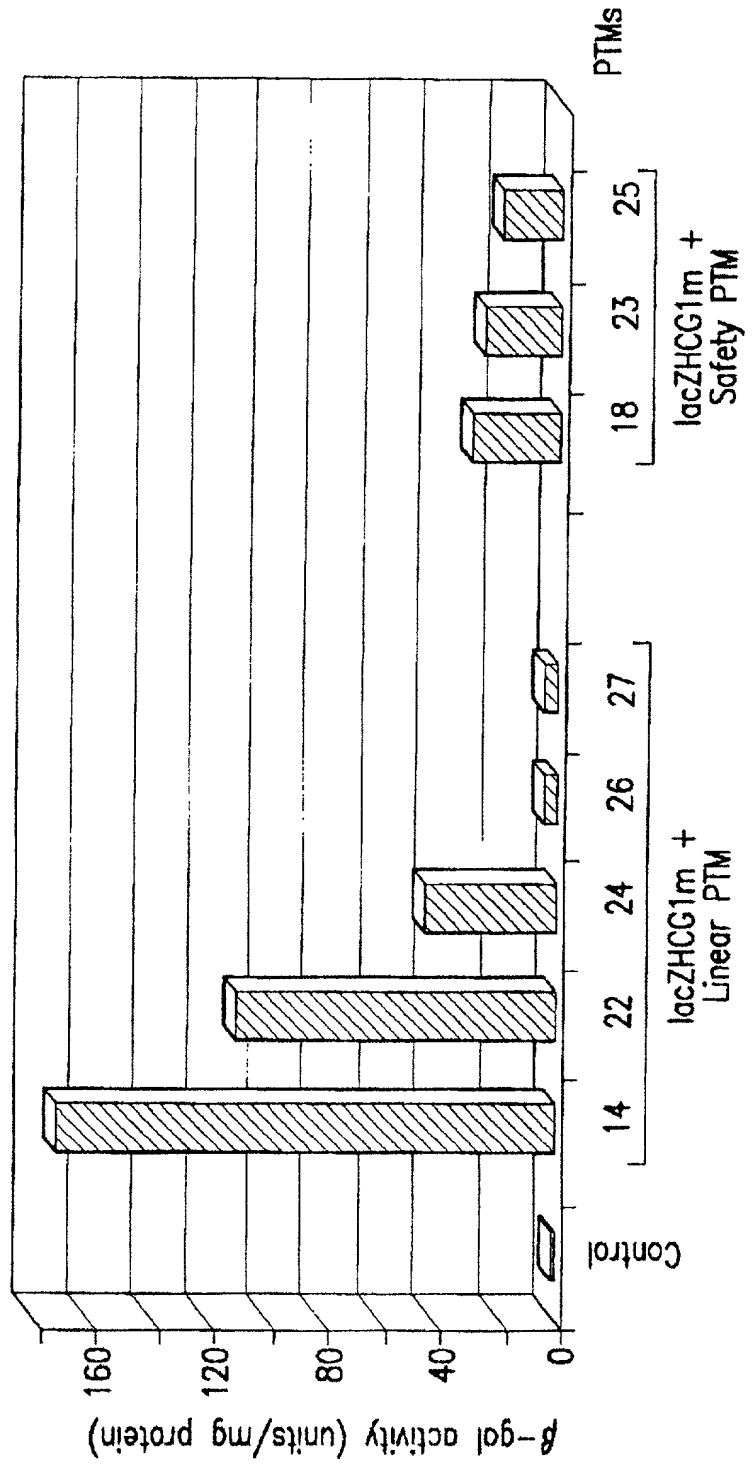


FIG.41C

## Exons

1-10

ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG  
 GATACAGACAGCGCCTGGAATTGTCAGACATATACCAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT  
 GGAAAGAGAATGGGATAGAGAGCTGCCTTCAAAGAAAAATCCTAAACTCATTATGCCCTTCGGCGATGTTTTTCTGG  
 AGATTTATGTTCTATGGAATCTTTTATATTTAGGGGAAGTCACCAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA  
 TAGCTTCCATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTATCTAGGCATAGGCTTATGCCCTCTCTTTAT  
 TGTGAGGACACTGCTCTACACCCAGCCATTTTTGGCCTTCATCAGATTGGAATGCAGATGAGAATAGCTATGTTTGT  
 TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTGTTAGTCTCCTTT  
 CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCAGATTTGCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT  
 CATGGGCTAATCTGGGAGTTGTTACAGCGCTCTGCCTTCTGTGGACTTGGTTTCTGATAGTCTTGGCCTTTTTTCAG  
 GCTGGGCTAGGGAGAATGATGATCAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAGAGCTTGTGATTACCTCAG  
 AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA  
 AACAGAACCTGAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTCTTT  
 GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGAAAAATATCACCACCATCTCATTCT  
 GCATTGTTCTGCGCATGGCGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA  
 CAAAATACAGGATTTCTTACAAAAGCAAGAATAAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG  
 AATGTAACAGCCTTCTGGGAGGAGGATTTGGGGAATTATTTGAGAAAGCAAAACAAACAATAACAATAGAAAACTT  
 CTAATGGTGATGACAGCCTCTTCTTCACTAATTTCTCACTTCTTGGTACTCCTGTCTGAAAGATATTAATTTCAAGAT  
 AGAAAGAGGACAGTTGTTGGCGTTCCTGGATCCACTGGAGCAGGCAAGACAGAGCTTGCTCATGATGATCATGGGCGAG  
TTAGAACAAGTGAAGGCAAGATCAACATTCCGGCCGATCAGCTTTTGCAGCCAATTGAGTTGGATCATGCCGGTA  
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCCCTCGGTGATTAAGGCCCTGCAGTTGGA  
GGAG

## Trans-splicing domain

GTAAGATATCACCGATATGTGCTAACCTGATTCGGGCCCTTCGATACGCTAAGATCCACCGG  
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG  
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATCTTCCACTGT  
GCTTAATTTTACCCTCTGAATTCCTATTTCTCCATAATCATATTACAACCTGAACTCTGGAATAAAACCCATCATT  
ATTAACCTATTATCAAAATCAGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I

153 bp BD underlined

GCTAGC-AATAATGACGAAGCGCGCCCTCAGCTCAGGATTCACCTGCCCTCCAATTATCATCCTAAGCAGAGTGATA

TTCTTAATTTGTAAGATTCTATTAACTCATTTGATTCAAAATATTTAAATACTTCCTGTTTCACCTACTCIGCTATGC

Sac II

AC-CCGCGG

FIG.43A

## Trans-splicing domain

AATAATGACGAAGCCGCCCTCAGGCTCAGGATTCACITGCCCTCCAATTATCATCCTAAGCAGAAGTGATATCTTA  
TTTGTAAGATTCTATTAACATTTGATTCAAAATATTTAAATACTTCCTGTTTCACTACTCTGCTATGCACCCGC  
GGAACATTATTATAACGTTGCTCGAATACTAAGTGGTACCTCTTCTTTTTTTTGATATCCTGCAG

## Exons 10-24

ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAGAATTTCATTCT  
 GTTCTCAGTTTTCTGGATTATGCCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTCCTATGATGAATATAGATA  
 CAGAAGCGTCATCAAAGCATGCCAAGTAGAAGAGGACATCTCCAAGTTTCAGAGAAAAGACAATATAGTTCTTGGAGAA  
 GGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTGTATT  
 TATTAGACTCTCCTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATTTGAAAGCTGTGTCTGTAACATGATGGC  
 TAACAAAAGTAGGATTTGGTCACTTCTAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT  
 AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAACTCATGGGATGTGATT  
 CTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCATCCTAACTGAGACCTTACACCGTTTCTCATTAGAAGGAGATGC  
 TCCTGTCTCCTGGACAGAAACAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGAAAAAAGCAAGAAATTCATT  
 CTCAATCCAATCAACTCTATACGAAAATTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT  
 CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATTTCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT  
 GATCAGCACTGGCCCCACGCTTCAGGCAAGGAGGAGCAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT  
 CAGAACATTCACCGAAAGACAACAGCATCCACAGAAAAGTGTCACTGGCCCCCTCAGGCAAACTTGACTGAACCTGGATA  
 TATATTCAGAAGGTTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT  
 TTTTGATGATATGGAGAGCATACCAGCAGTCACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA  
 ATTTTGTGCTAATTTGGTGTCTAGTAATTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTCTGTGGCTCCTTGGAA  
 ACACTCCTCTTCAAGACAAAGGAATAGTACTCATACTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC  
 GTATTATGTGTTTTACATTACGTGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG  
 GATACTCTAATCACAGTGTGCAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCTCA  
 ACAAGTTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAGATATAGCAATTTGGATGACCTTCTGCCTCTTACCAT  
 ATTTGACTTCATCCAGTTGTTATTAATTTGATTTGGAGCTATAGCAGTTGTCCAGTTTTACAACCTACATCTTTGTT  
 GCAACAGTGCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAAACCTCACAGCAACTCAAACAAGTGG  
 AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTGTTACAAGCTTAAAGGACTATGGACACTTCGTGCCTTCGGAGC  
 GCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTAACCTGTCAACACTG  
 CGCTGGTTCCAAATGAGAAATAGAAATGATTTTGTATCTTCTTCATTGCTGTACCTTCATTTTCAACAACAG  
 GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGCTGTAAACTC  
 CAGCATAGATGTGGATAGCTTGATGGATCTGTGAGCCGAGTCTTTAAGTTCAATTGACATGCCAACAGAAGGTAAACCT  
 ACCAAGTCAACCAAAACCATACAAGAAAGGCAACTCTCGAAAGTTATGATTATTGAGAATTACACAGTGAAGAAAGATG  
 ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA  
 GAACATTTCTTCTCAATAAGTCTCGCCAGAGGCTGGGCTCTTGGGAAGAACTGGATCAGGAAGAGTACTTTGTTA  
 TCAGCTTTTGTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCATAACTTTGCAAC  
 AGTGGAGGAAACCTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCTGGAACATTTAGAAAAAAGTTGGATCCCTA  
 TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCAGATGAGGTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG  
 AAGCTTGACTTTGTCTTGTGGATGGGGCTGTGTCTTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG  
 TTCTCAGTAAGCGGAAGATCTTGCTGTGTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAATAATTAGAAG  
 AACTCTAAAACAAGCATTGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA  
 TTTTGGTCTATAGAAGAGAACAAAGTGGGCACTACGATTCCATCCAGAAAGTGTGAACGAGAGGAGCCTCTTCCGGC  
 AAGCCATCAGCCCTCCGACAGGTGAAGCTTTTCCCACCGGAAGCTCAAGCAAGTGAAGTCTAAGCCCCAGATTGC

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATCATTAG

FIG.43B

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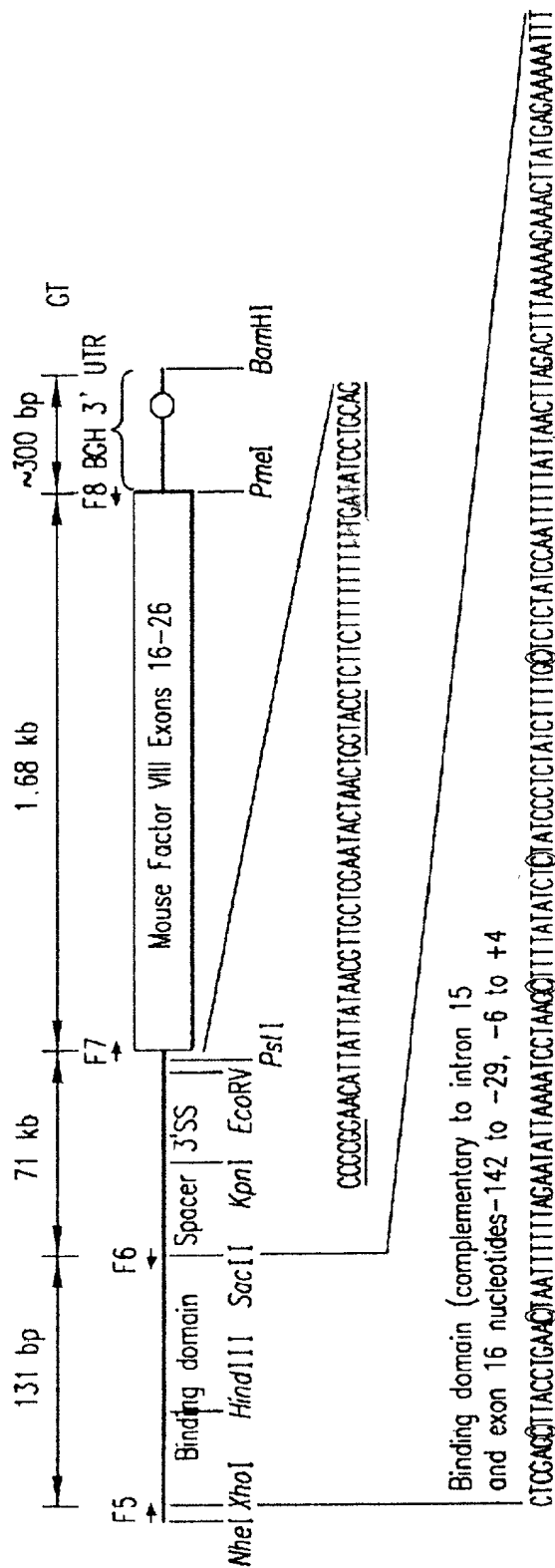


FIG. 44A



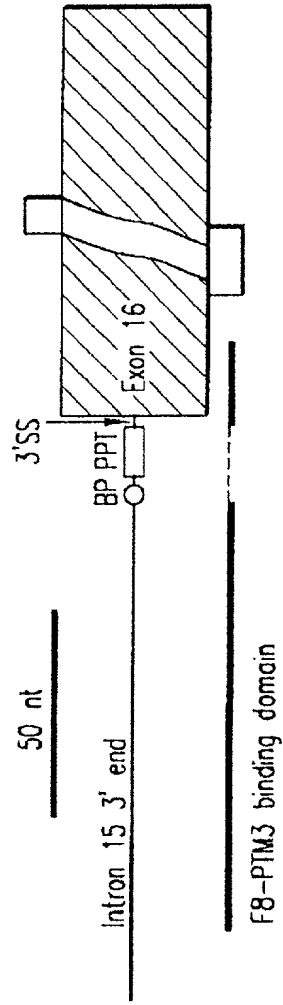


FIG.44B



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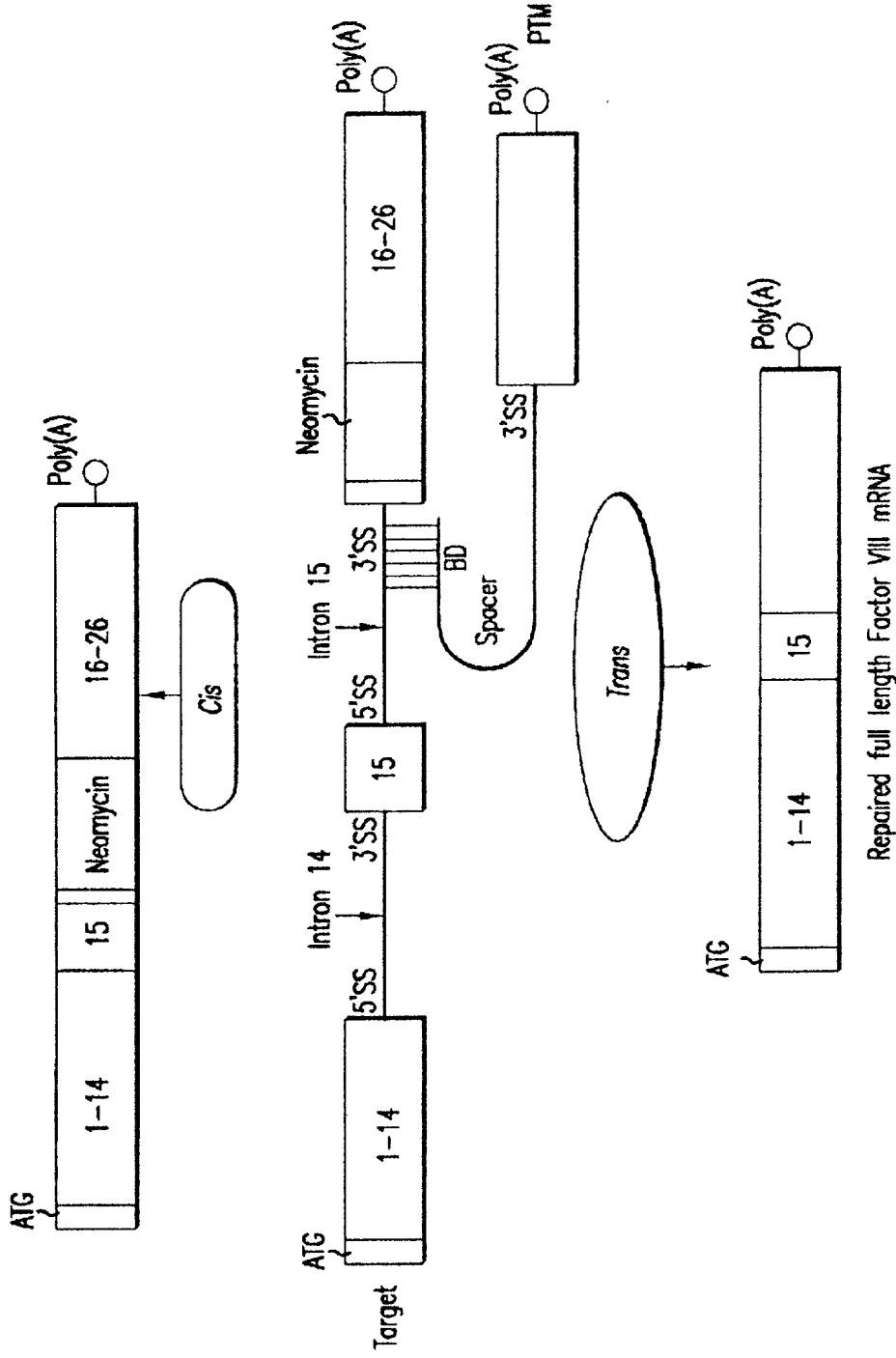
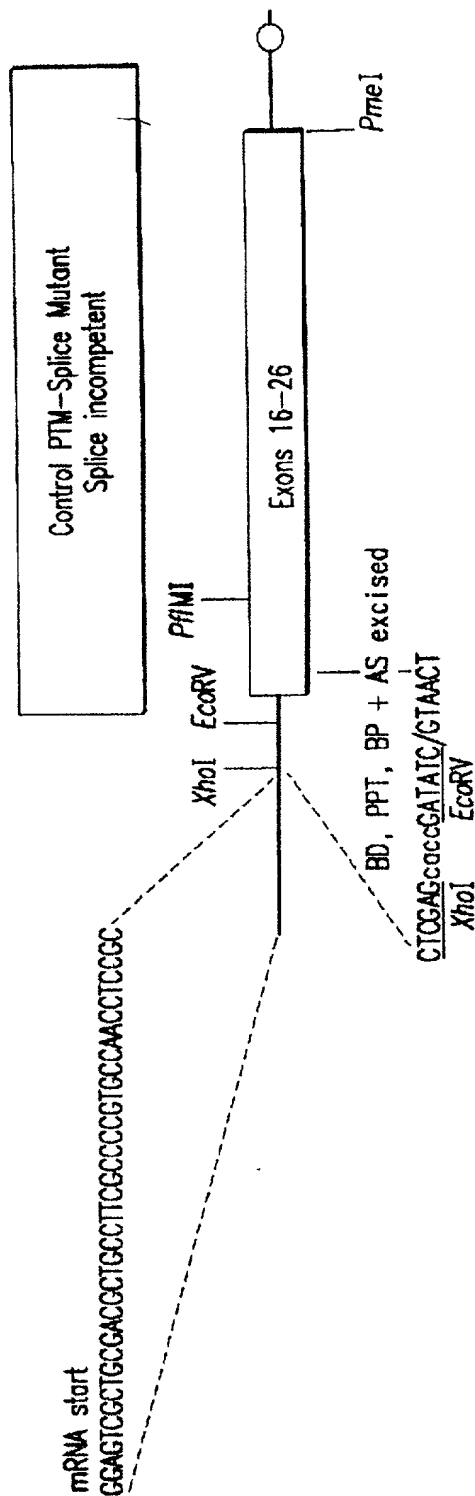


FIG.44D



Method:  
Excise TSD and part of exon 16 with  
*XhoI* and *PflMI* and ligate in a PCR product that:  
1) eliminates the TSD and splice acceptor site  
2) inserts *EcoRV* adjacent to exon 16  
3) restores the coding for exon 16

FIG.45

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Repair of Factor VIII  
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice  
after IV PTM-FVII intraportal infusion  
(100  $\mu$ gDNA)(n=3)

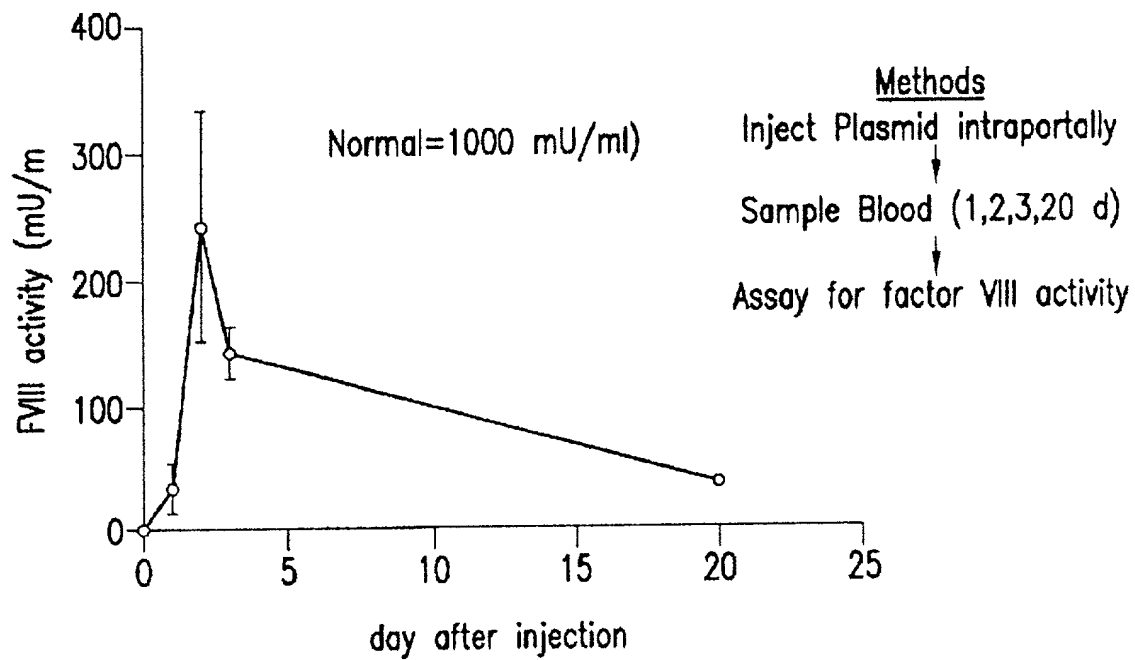


FIG.46

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

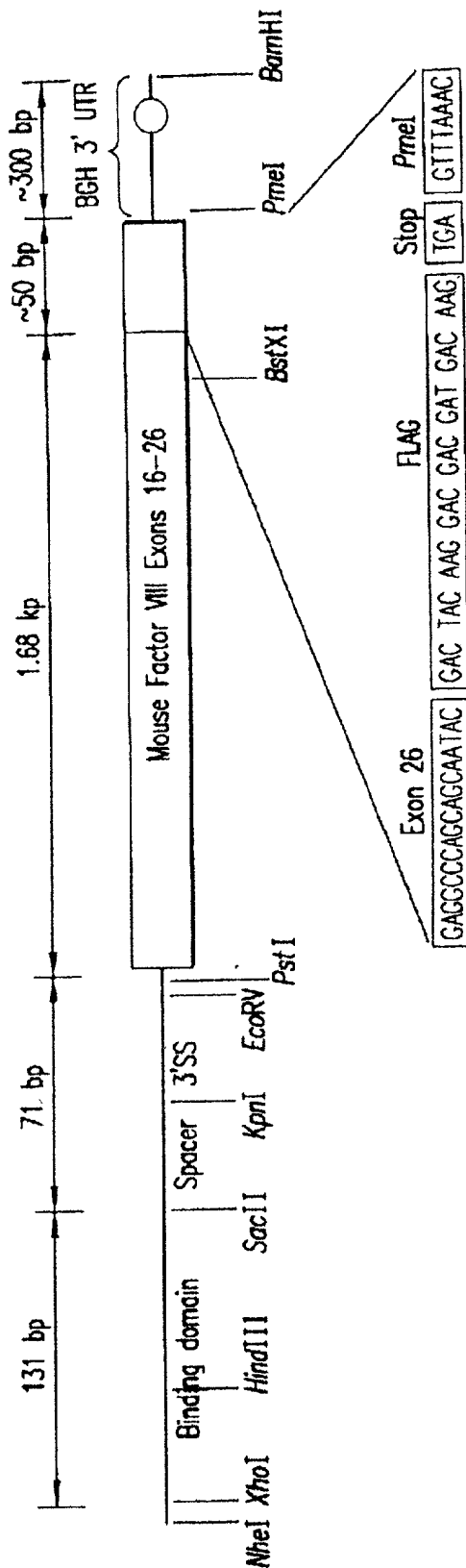
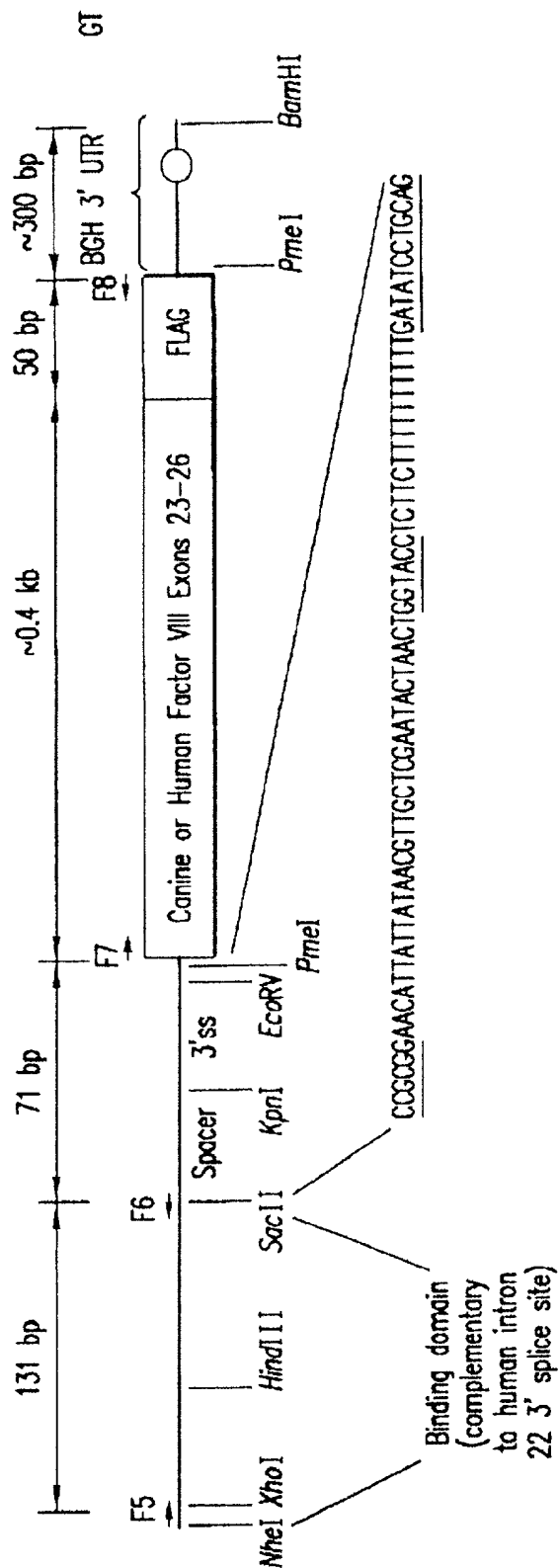


FIG.47A



FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG. 47B

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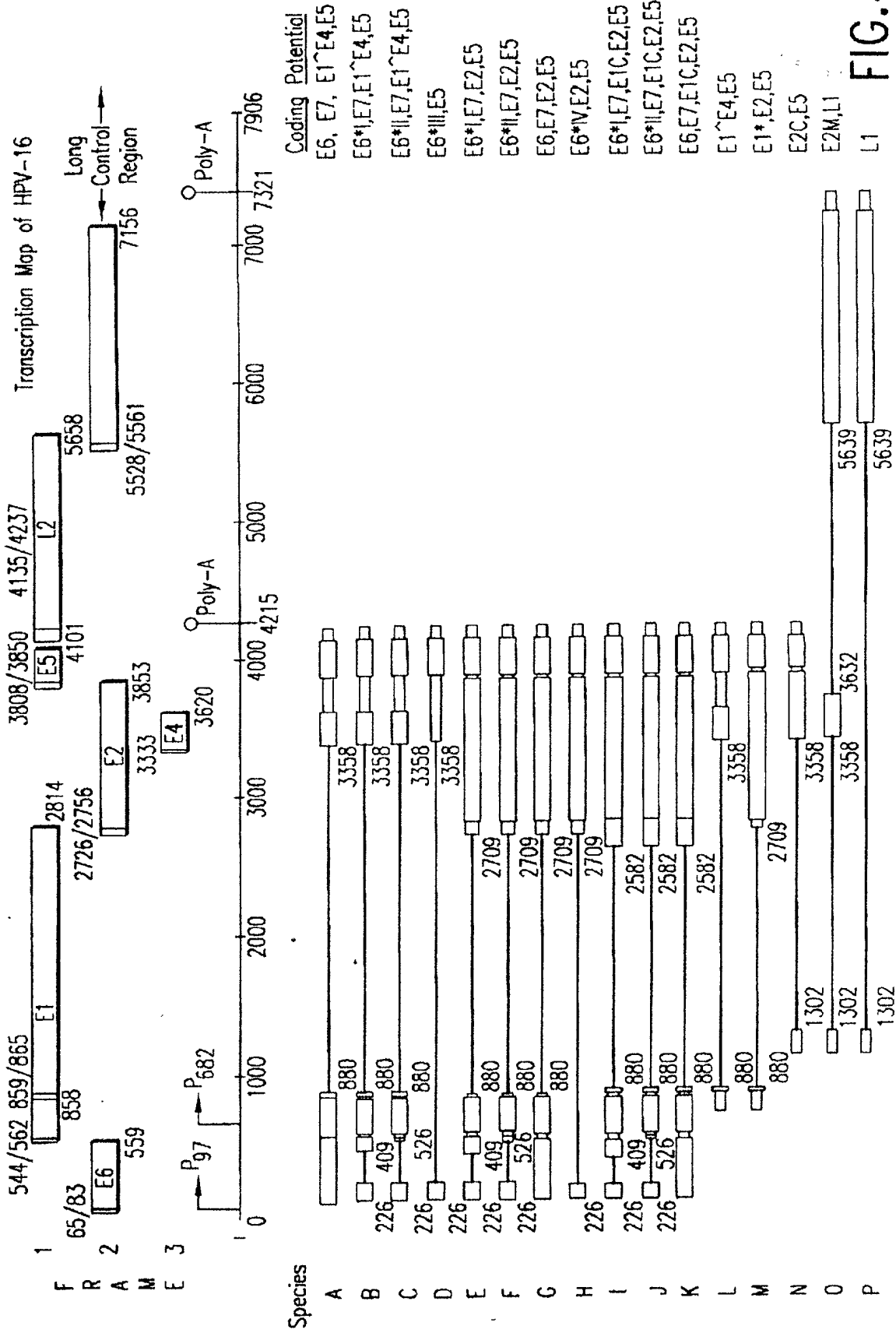
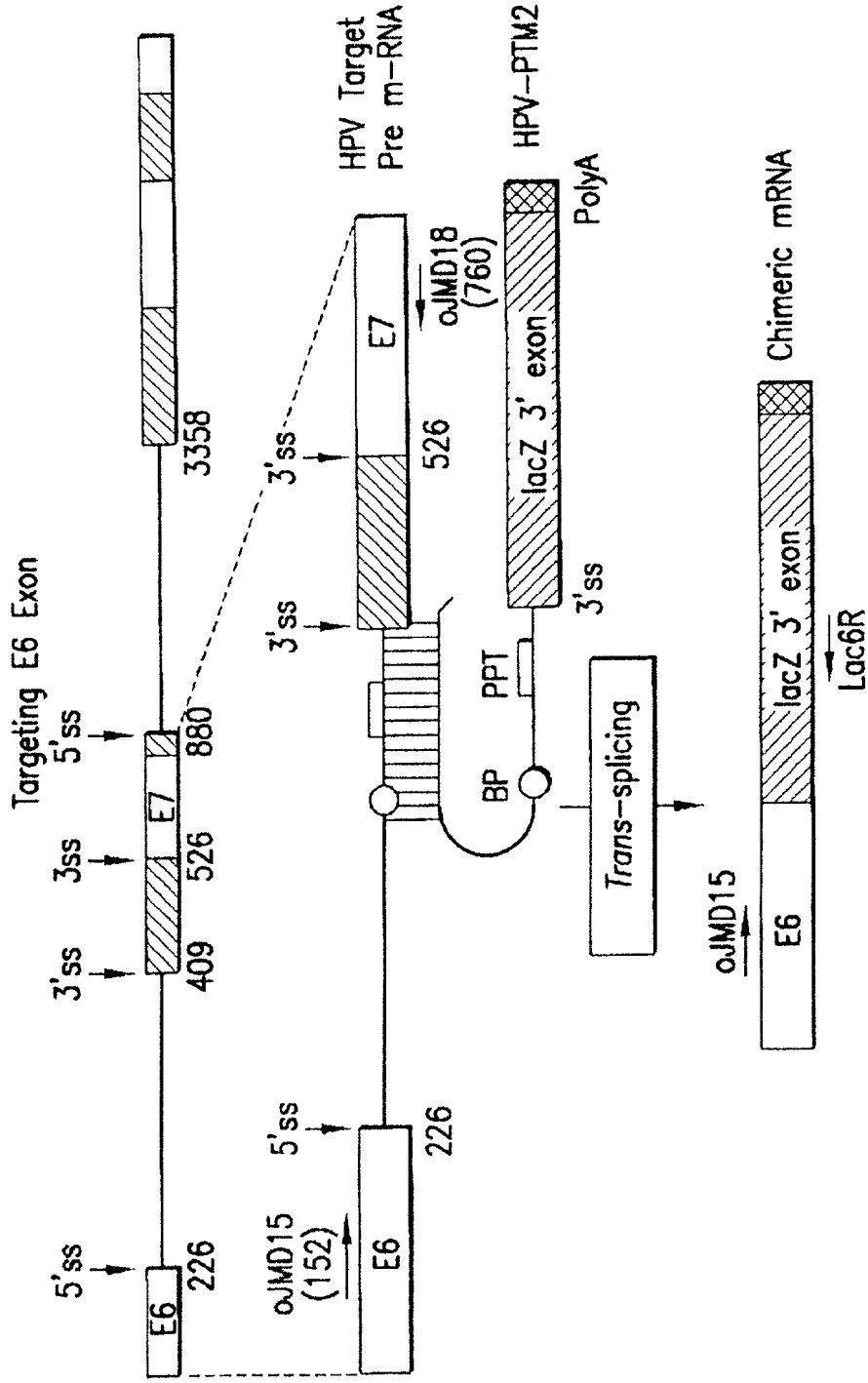


FIG.48

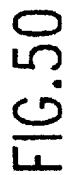


# SMART Strategy to Disrupt the Expression of Human Papillomavirus Type 16



SMART Strategy by 3' Exon Replacement: Schematic diagram of HPV-PTM2 binding to the 3' splice site of the HPV type 16 target pre-mRNA

FIG.49



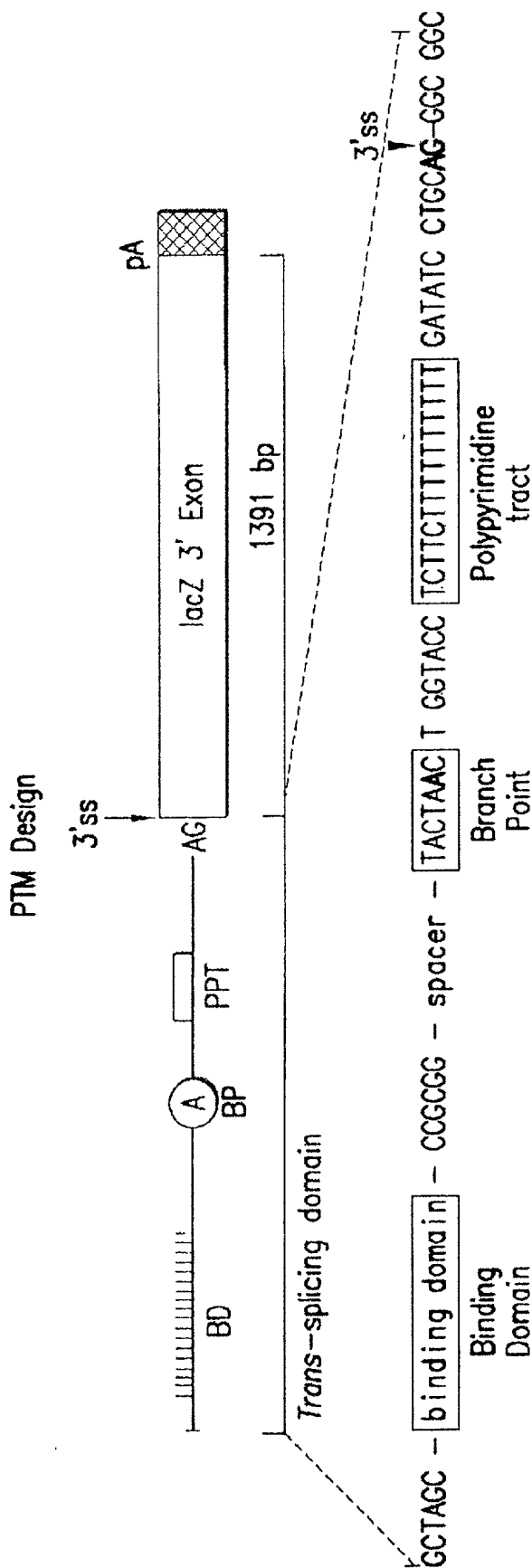


FIG.51

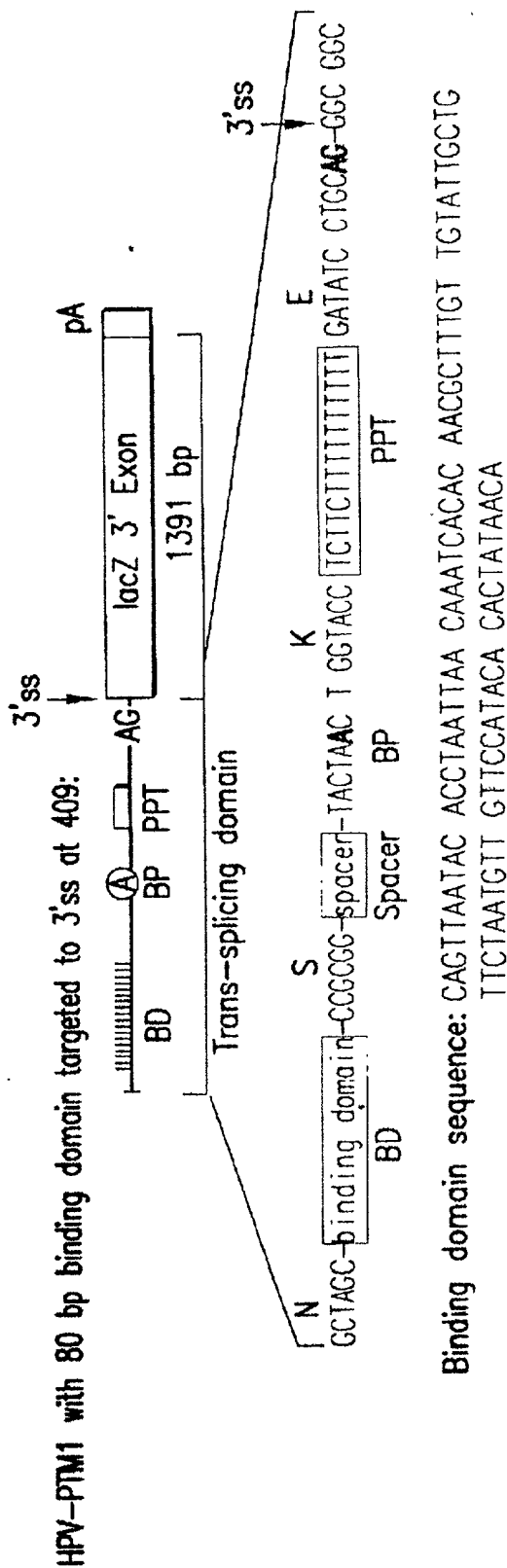
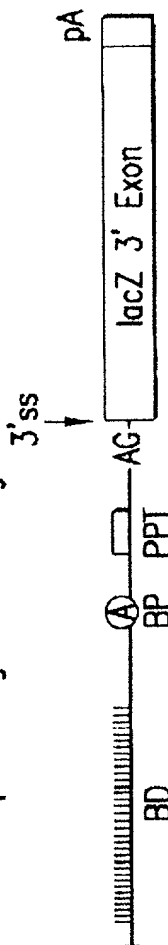


FIG.52A

HPV-PTM2 with 149 bp binding domain targeted to 3'ss at 409:

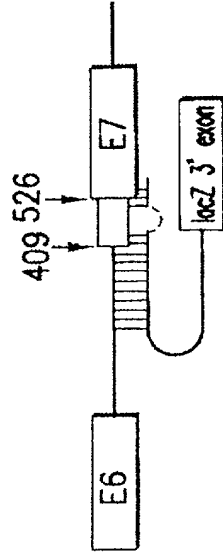


Binding domain sequence: CAGTTAATAC ACCTAATTAA CAAATCACAC AACGCTTIGT TGTATTCGICG  
TTCTAATGTT GTTCCATACA CACTATAACA ATAATGCTCA TACTACACTAA  
TTTTAGAATA AAACITTTAA CATTATATCAC ATACAGCATA TCGATTCCC

FIG.52B

# Binding Domains of HPV-PTM3 and 4

HPV-PTM3 Binding domain (covers both 3'ss at 409 and 526; has 53 bp bubble)  
 GATGATCTGCAACAAGACATACATCGACCGTCCA(53 nt bubble)CTTCAGGACACAGTGGCTTTTGAC  
 AGTTAATACACCCTAATTAAACAAATCACACAACGGTTTGTTGTTATTCAGTTCTAATGTTGTTCCATACACACTA  
 TAACAAT



HPV-PTM4 Binding domain (covers both 3'ss at 409 and 526; has 76 bp bubble)  
 GATGATCTGCAACAAGAC(76 nt bubble)GACACAGTGGCTTTTGACAGTTAATACACCCTAATTAAACAAATC  
 ACACAACGGTTTGTTGTTATTCAGTTCTAATGTTGTTCCATACACACTATAACAAT

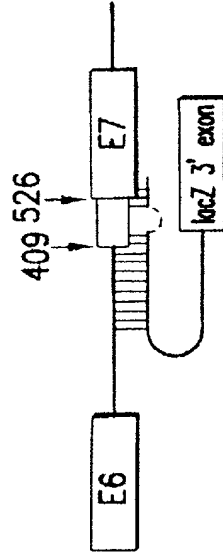
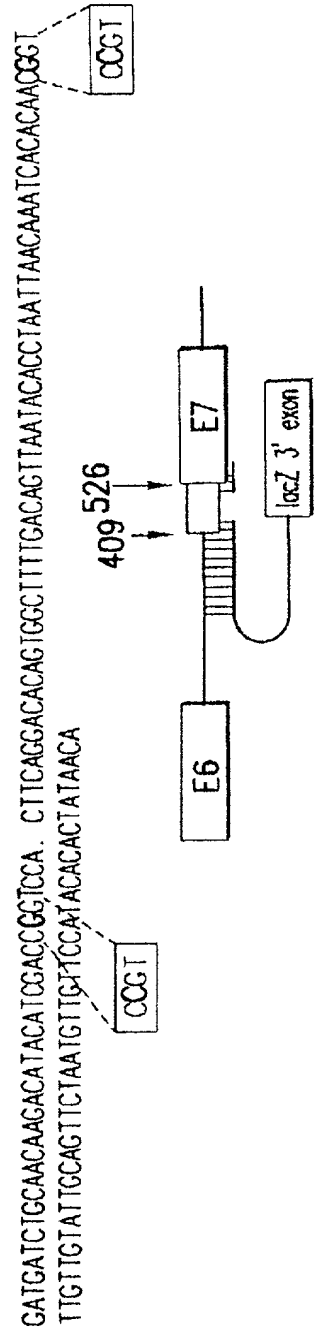


FIG.53

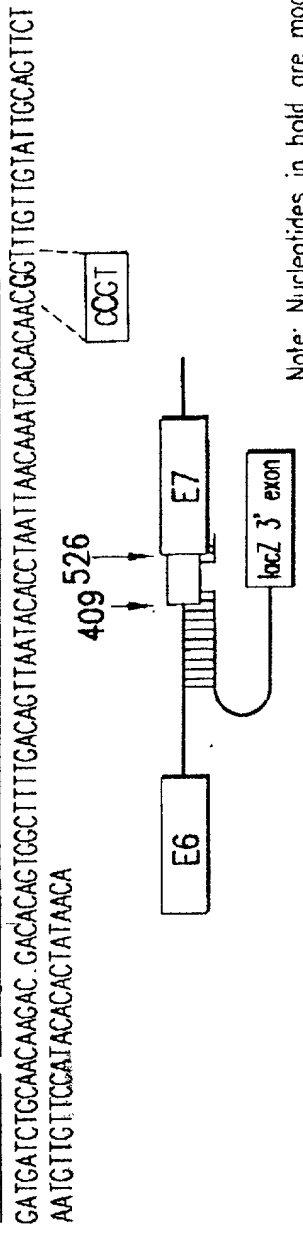
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### HPV-PTM5 and 6

**HPV-PTM5**, Binding domain (140 nt, has **53 nt bubble**, covers 3'ss at position 409 and 526)



**HPV-PTM6**, Binding domain (117 nt, has **76 nt bubble**, covers 3'ss at position 409 and 526)



Note: Nucleotides in bold are modified to prevent PTMs cryptic splicing

FIG.54

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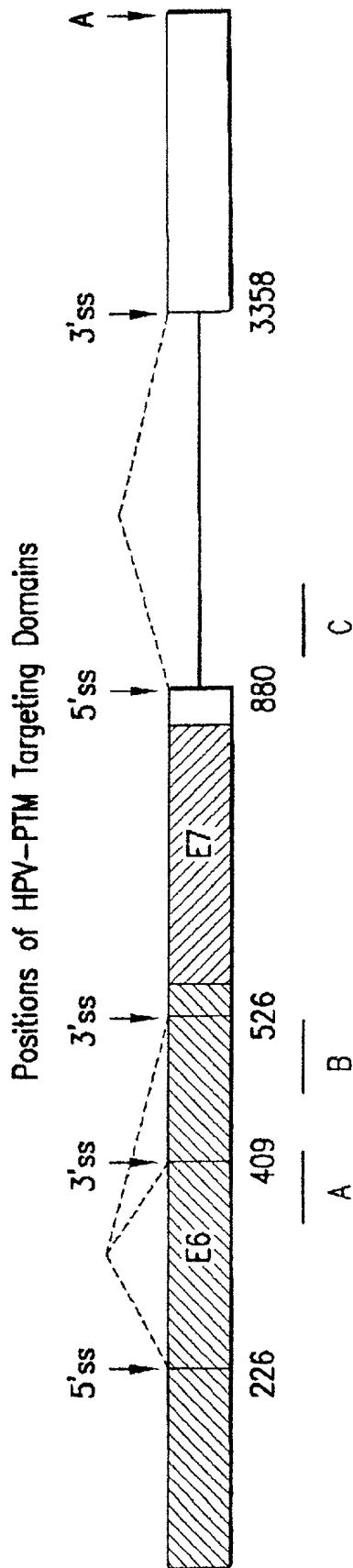
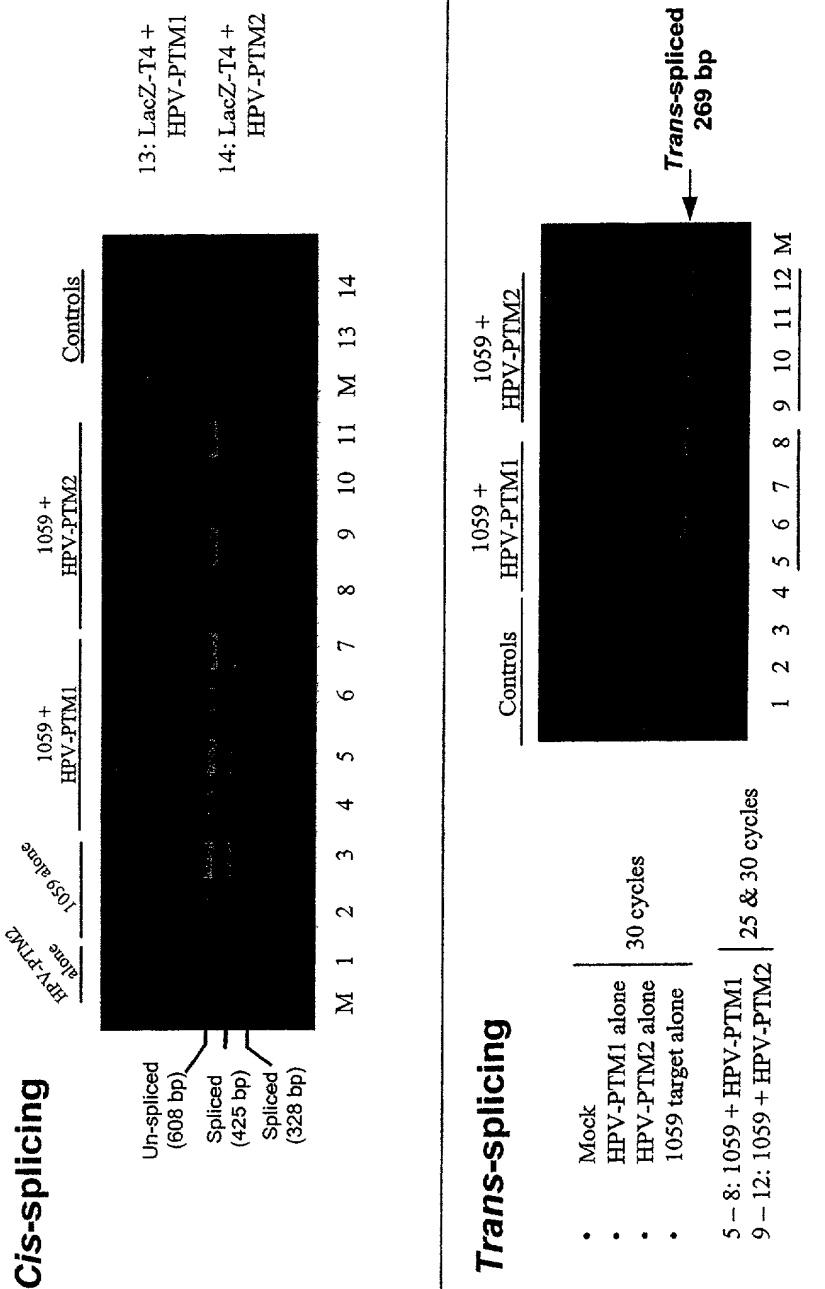


FIG.55

80 2 91

# Trans-splicing Efficiency of HPV-PTMs in 293T Cells



RT-PCR Analysis of total RNA

INTRON

FIG. 56



Trans-splicing between target pre-mRNA and  
PTM is accurate (293T cells)

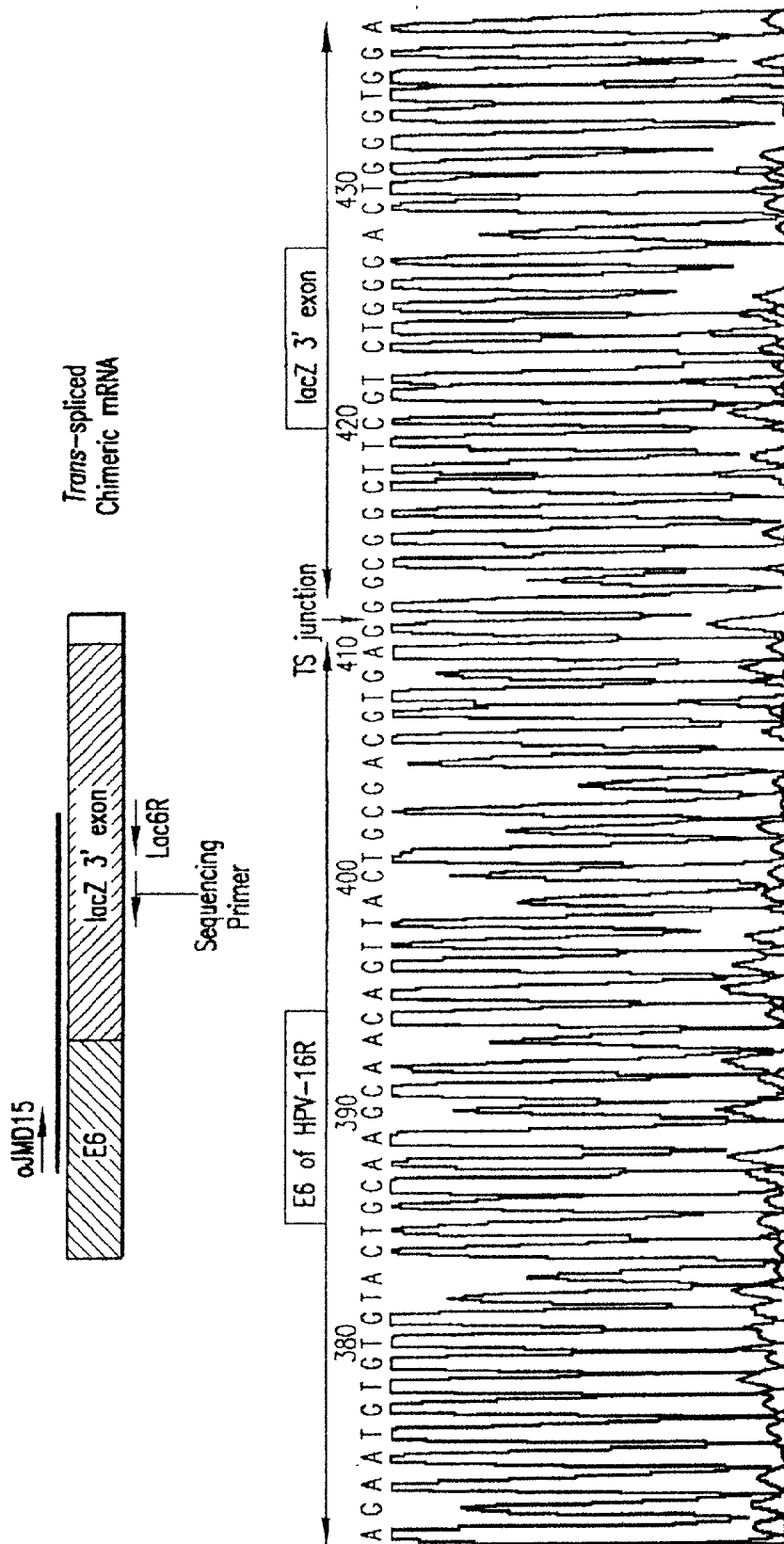
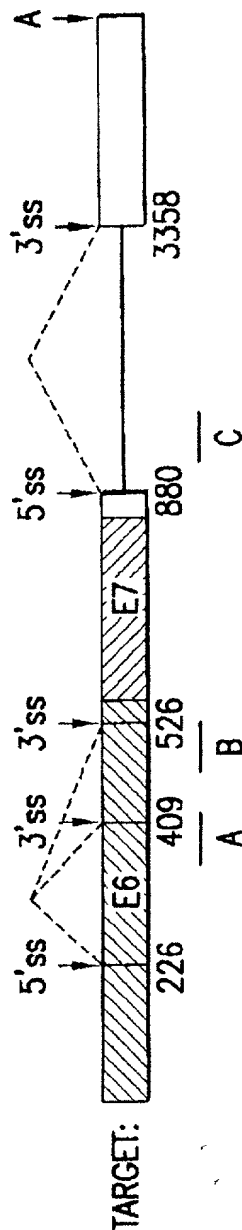


FIG.57

Trans-splicing in 293 Cells (Co-transfections)



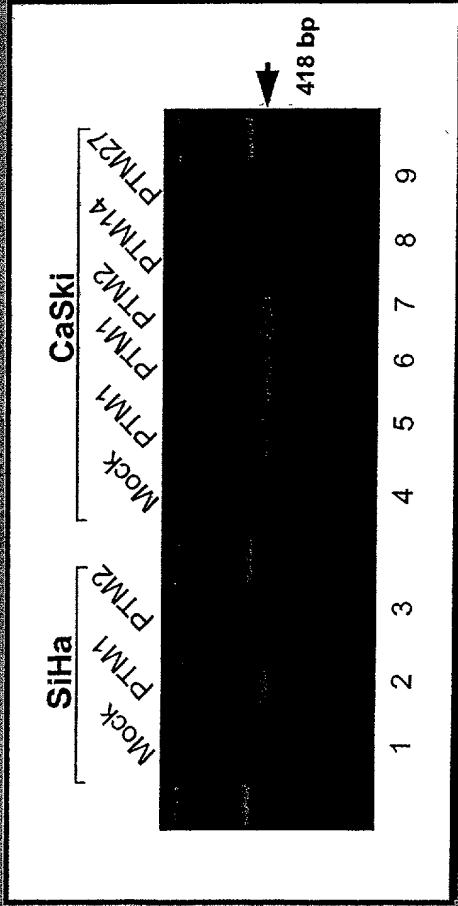
PTM	Binding Domain		% trans-spliced	
	Region	Size (nt)	226 sd	880 sd
HPV-PTM1	A	80	69	0.6
HPV-PTM2	A	149	45	0.9
HPV-PTM5	A+B	140	55	0.8
HPV-PTM5ΔBP/PPT	A+B	140	0.5	0.2
HPV-PTM6	A+B	117	59	1
HPV-PTM8	C	104	7	37
HPV-PTM9	C	174	14	22
CF-PTM27	CF intron	411	0	0

Quantification of Trans-splicing efficiency using real-time QRT-PCR

FIG.58

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# Trans-splicing into Endogenous HPV Pre-mRNA Target in SiHa & CaSki Cells



SiHa: Single copy  
CaSki: ~400-500 copy/II

Trans-spliced  
Chimeric mRNA

E6  
lacZ-3'exon

RT-PCR Analysis of total RNA

## RT-PCR Conditions

- Total RNA: 400 ng/rxn
- Primer's: oJMD15 + Lac16R
- # Cycles : 35
- Expected product : 418 bp

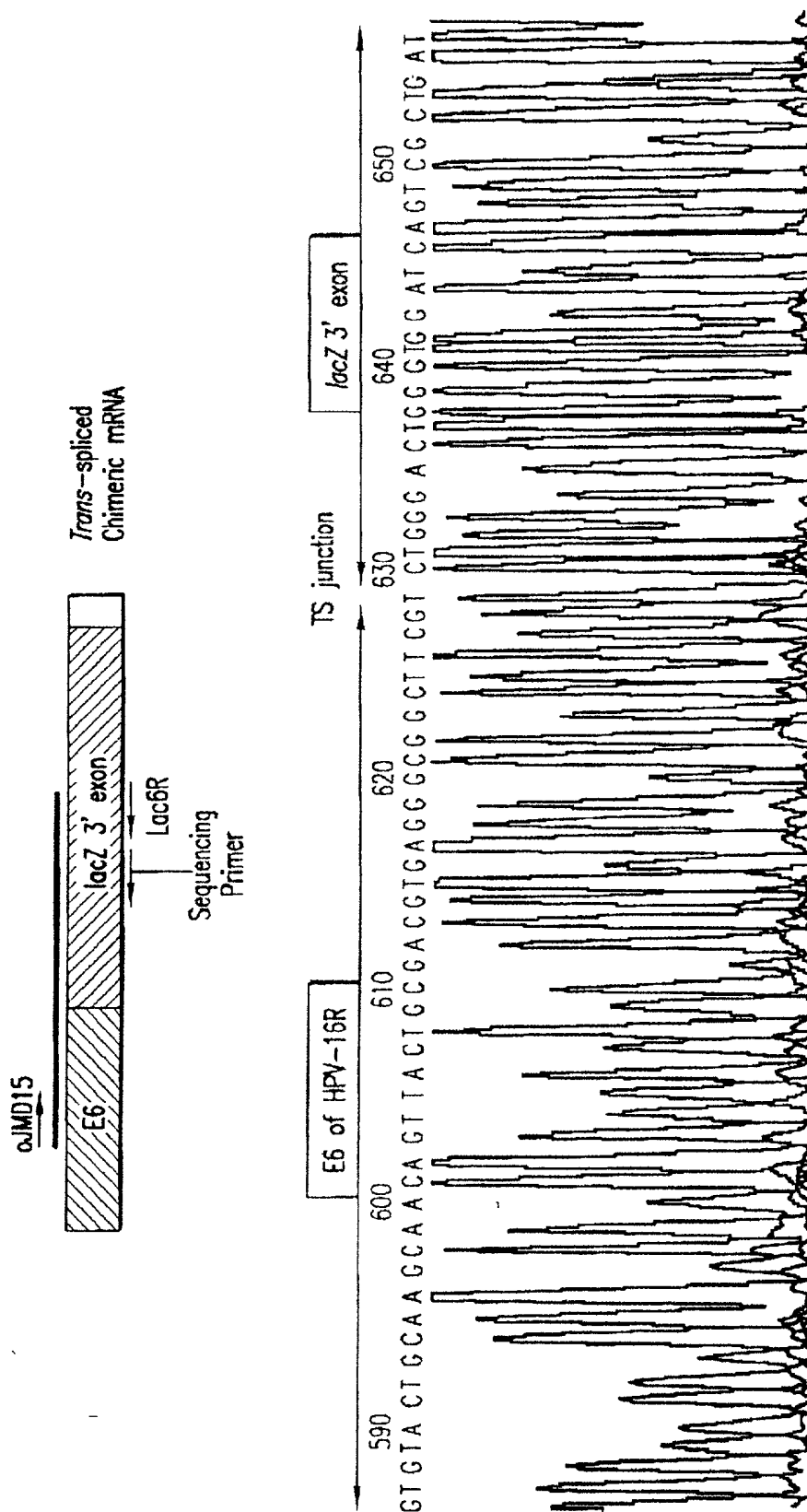
## Details

- PTM1, PTM2 : HPV targeted, specific
- PTM14 : CF targeted, non-specific, has 23 bp BD
- PTM14 : CF targeted, non-specific, has 411 bp BD

INTRON

FIG. 59

# Accurate Trans-splicing of HPV-PTM1 in Si Ha Cells (Endogenous target pre-mRNA)



Quantification of trans-splicing efficiency using real-time QRT-PCR

FIG.60

*Trans*-splicing in SiHa Transfections  
(Endogenous target)

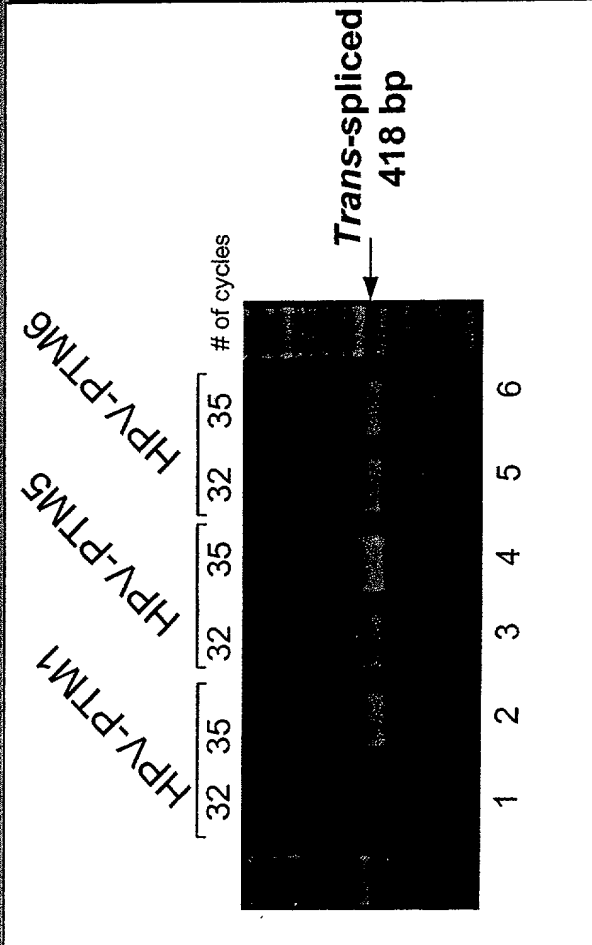
<u>PTM</u>	<u>% trans-spliced</u>
pcDNA3.1	0
HPV-PTM1	0.16
HPV-PTM5	0.12
HPV-PTM6	0.11
CF-PTM27	0

Quantification of *trans*-splicing efficiency using real-time QRT-PCR

FIG.61

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# **Trans-splicing Efficiency of HPV-PTM1, 5, & 6 in SiHa Cells**



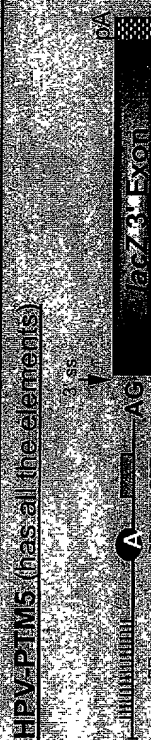
- SiHa cells transfected with 1.5 µg plasmid DNA, LipoPlus
- RNA isolated after 48 hr
- Total RNA: 500 ng/Rxn
- Primers: oJMD15 + Lac16R
- Expected product: 418 bp

1, 3, 5, : 32 cycles  
2, 4, 6, : 35

INTRONN

FIG. 62

## Deletion of polypyrimidine tract abolishes *trans*-splicing



### Methods:

- SiHa cells transfected with 1.5  $\mu$ g of plasmid DNA
  - Total RNA isolated after 48 hr and analyzed by RT-PCR (30 cycles)
- Primers: oJMD15+Lac6R  
Expected product: 269 bp
- Lanes 1 & 2: RNA from cells transfected with HPV-? PPT (mutant); No trans-splicing detected

Lanes 3 & 4: RNA from cells transfected with HPV-PTM5 plasmid; trans-splicing Detected (269 bp product)

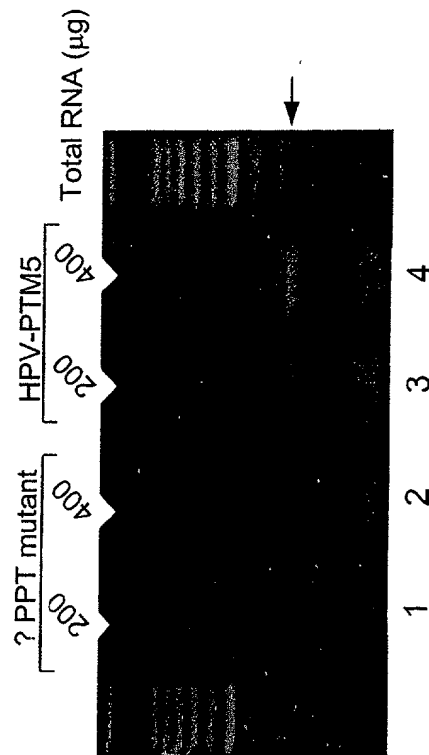
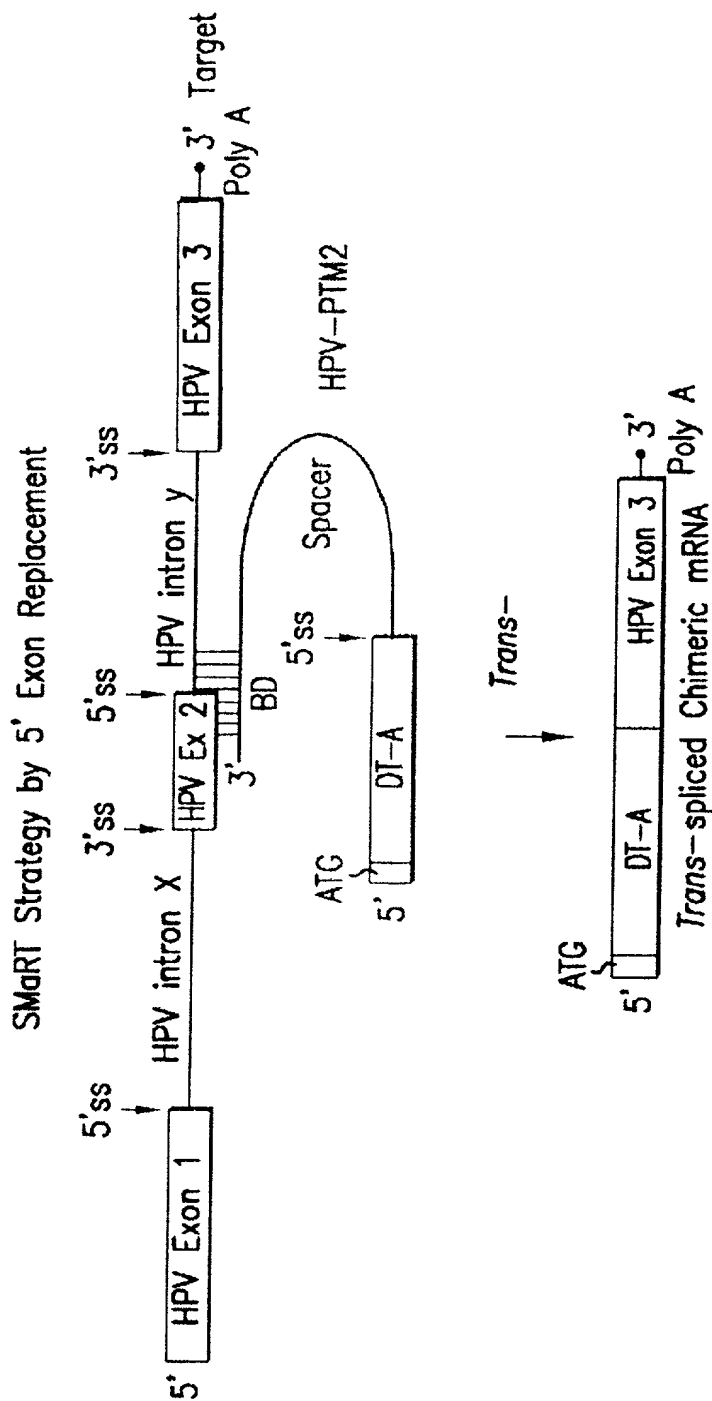


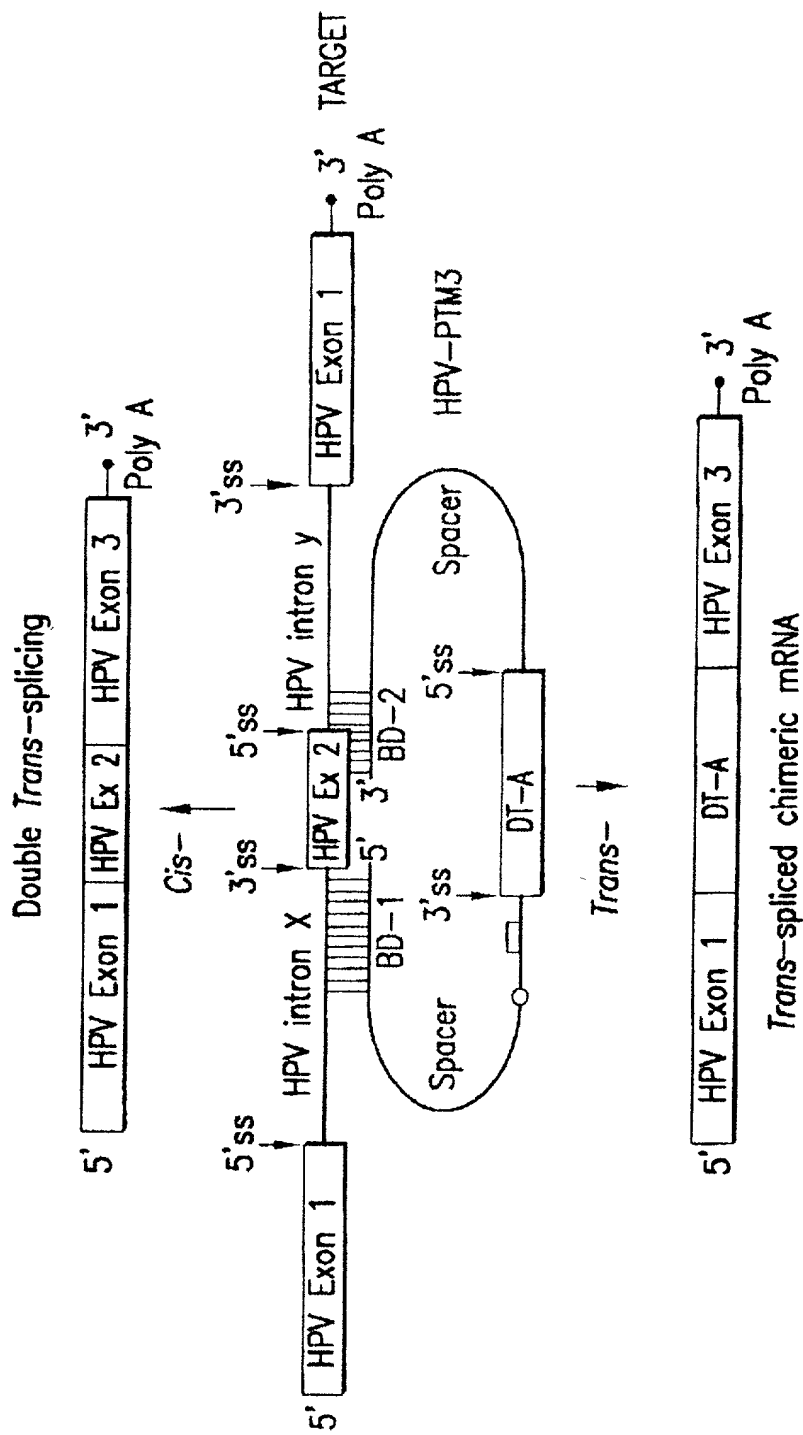
FIG. 63



Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

FIG.64





Schematic diagram of a double Trans-splicing PTM binding to the 3' and 5' splice sites of the HPV mini-gene target

FIG.65

SMaRT Strategy by 3' Exon Replacement: Schematic diagram of a PTM binding to the 3' splice site of the HPV mini-gene target

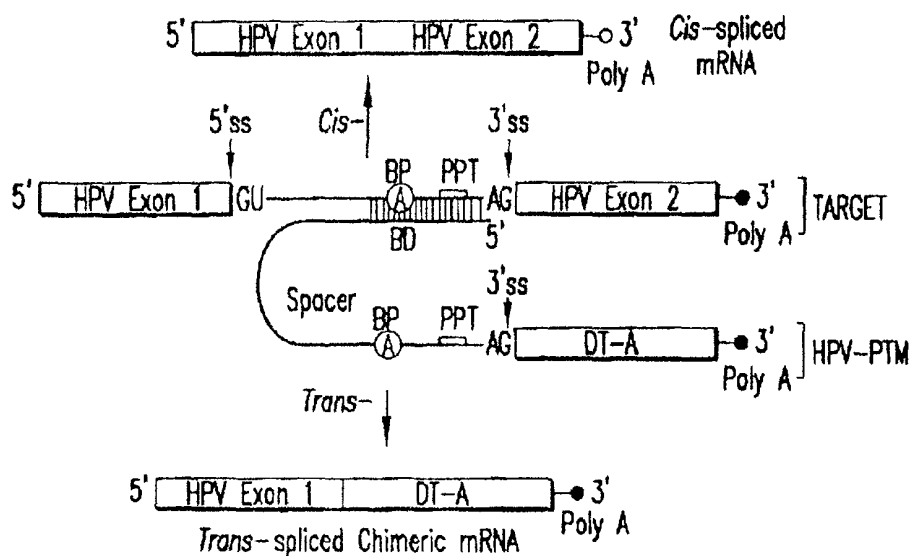


FIG.66A

SMaRT Strategy by 5' Exon Replacement: Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

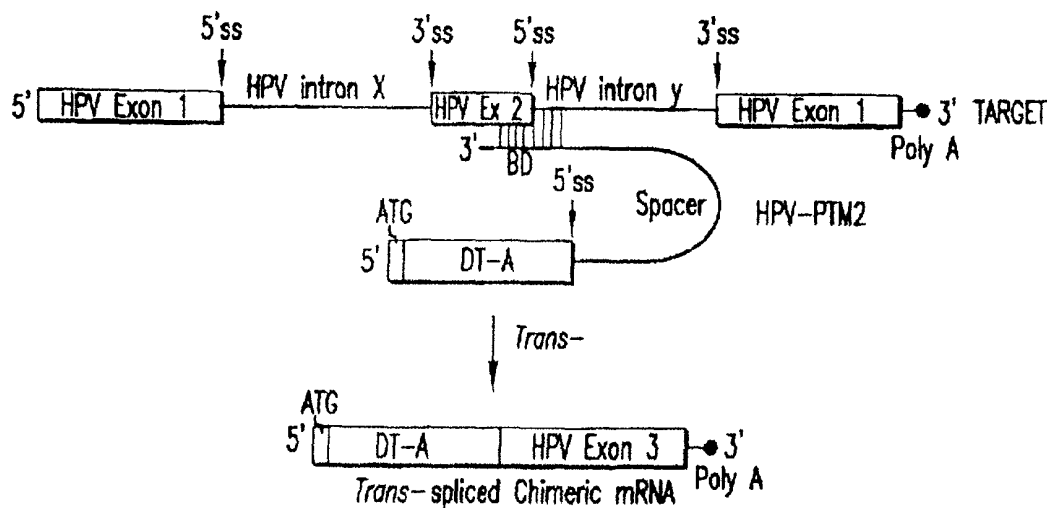


FIG.66B

HPV-PTM3 (For internal exon replacement)

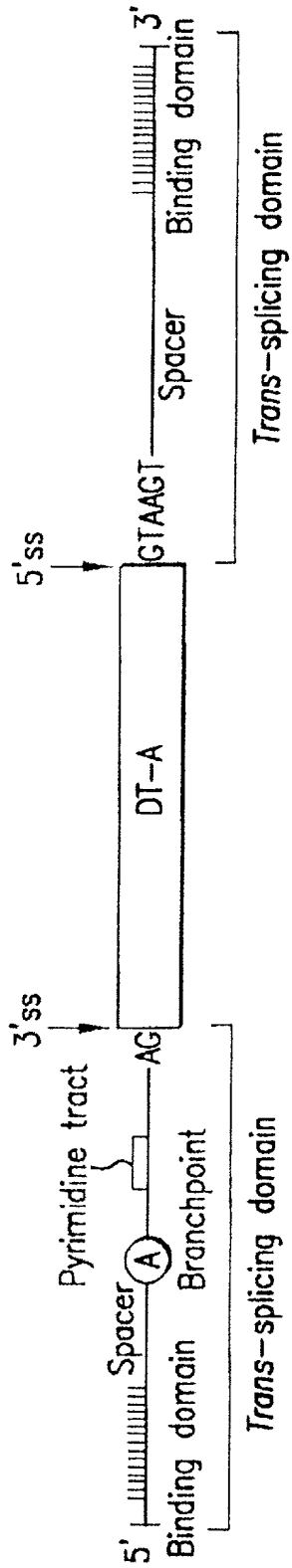


FIG.67

91 8 91